MEASLES AND RUBELLA SEROLOGICAL SURVEILLANCE IN ZAMBIA: DESIGN, IMPLEMENTATION, AND ANALYSIS

by

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Abstract

Measuring population immunity to infectious diseases is an important metric to assess the potential for outbreaks and target interventions. Vaccination coverage is typically used to approximate population immunity levels, but the most direct measure is to collect biospecimens and test antibody levels in the population. This method of conducting serological surveillance can monitor progress towards measles and rubella elimination and flag immunity gaps to target for vaccination. Serosurveys have been conducted for both measles and rubella viruses, primarily in developed countries due to the costs, logistics, laboratory requirements, and analysis techniques that make serological surveillance complex.

This dissertation addresses serosurvey design topics including community acceptance, cost estimates, and data analysis issues when estimating seroprevalence from different survey designs. We use the context of two serosurveys conducted to monitor population immunity before and after a measles-rubella vaccination campaign in Zambia. First, we evaluated barriers and facilitators to serosurvey participation using quantitative and qualitative data. Qualitative data from data collectors who participated in a measles-rubella serosurvey and caregivers at health facilities identified interpersonal relationships and serosurvey design issues as key barriers and facilitators to participation. Provision of information ahead of the serosurvey to answer questions related to blood collection and communicating through a trusted source, such as the community health worker, were particularly important.

In the second aim, we captured the cost of implementing serosurveys. We estimated that up to two-thirds of costs could be saved by nesting a serosurvey in an already planned vaccination coverage survey. Identifying what contributes to serosurvey costs can help identify ways to reduce cost to make them more accessible to low- and middle-income countries.

Finally, in the third aim we evaluated the impact of the vaccination campaign in Southern province, Zambia. The pre and post-campaign serosurveys demonstrated significant increases in seroprevalence of antibodies to measles and rubella, highlighting the impact of the vaccination campaign. Comparing the serosurveys required survey weighting and direct adjustments to account for selection bias, specimen types and laboratory test kits. Characterizing the acceptability, cost and analytic challenges can help improve the design and implementation of serological surveys to meet programmatic goals.

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Background

In 2012, the Global Strategic Plan for Measles and Rubella established goals of reaching elimination of measles in at least five World Health Organization (WHO) regions and rubella in three regions by 2020. The Region of the Americas is the only region thus far to achieve the elimination of rubella in 2015 (Pan American Health Organization, 2016). While elimination of measles was reached in the Americas in 2016, this status was lost in 2018 due to re-established transmission in Venezuela, highlighting the fragility of measles elimination status (Pan American Health Organization, 2018). The strategy for elimination includes high vaccination coverage, laboratory surveillance, monitoring and evaluation, outbreak response, and community engagement. In order to design the elimination program, countries should set target levels of immunity, establish current immunity profiles, and identify strategies to increase immunity to reach the target. Serological surveillance can help to establish immunity profiles and identify population immunity gaps, which can be targeted for vaccination to ensure herd immunity thresholds are met so virus transmission can be interrupted (Trentini, Poletti, Merler, & Melegaro, 2017).

Measuring population immunity

Population immunity is the percentage of people in a given area protected against a disease. There are primarily three data sources ued to estimate population immunity: vaccination coverage, surveillance, and serological surveillance.

Population immunity profiles are often inferred from vaccination coverage data; however, these data can be of poor quality. Vaccination programs in low and middle-income

countries estimate vaccination coverage through: (1) immunization registries and routine administrative reporting; and (2) household surveys (Travassos et al., 2015). The best estimates of vaccination coverage for low and middle-income countries come from the WHO and United Nations Children's Fund (UNICEF) immunization coverage estimates which incorporate both administrative and survey data to establish one estimate per country on an annual basis. However, there are several data quality issues with routine administrative reporting. Administrative vaccination coverage has been reported over 100% in some districts, and has been found to not match survey data (MacNeil, Lee, & Dietz, 2014). Biased reporting can also occur during routine administrative reporting due to duplications of vaccination records, not accounting for private providers, and administrative or organizational changes such as redistricting. Additionally, immunization registries measure only the numerator, which is the number of children who have been vaccinated and could potentially count the same child vaccinated twice as different children. Immunization registries also do not account for denominator issues such as incomplete birth registration or migration.

Given the issues with routine administrative data, WHO and UNICEF incorporate survey data into their estimates of vaccination coverage. Two large multi-purpose surveys that are conducted on a routine basis are the Demographic Health Survey (DHS), supported by the United States Agency for International Development, and the Multiple Indicator Cluster Survey (MICS) supported by UNICEF. Both surveys are multi-stage cluster surveys with standardized questionnaires and field workers who collect data. Validation studies have suggested that DHS and MICS survey instruments had 44% sensitivity and 38-49% specificity for measuring measles containing vaccine (MCV) coverage (Liu et al., 2013). The main potential sources of error in population-based surveys measuring vaccination coverage include: sampling error; selection bias

due sampling issues; information bias due to poor field procedures, home-based records and inaccurate verbal history; and data entry or missing data (Cutts, Izurieta, & Rhoda, 2013). Other disadvantages of these surveys are that they are costly, require up to a year of planning, and are not generally designed to measure district-level coverage.

Neither routine administrative data nor household surveys directly measures population immunity. Because vaccination coverage is only a proxy measure for population immunity, it can be biased. In the absence of wild-type virus circulation, population immunity will generally be lower than vaccination coverage, as only "effective" vaccinations, which induce immunity in recipients, will be measured (Colson, Zuniga-Brenes, et al., 2015; Ibrahim, Abdallah, Saleh, Osterhaus, & De Swart, 2006). If multiple doses are offered, the second dose may be disproportionately given to individuals who already have a first dose of vaccine, thus not necessarily improving population immunity (Lessler et al., 2011). If there is a large outbreak of measles or rubella, population immunity would be expected to be higher than reported vaccination coverage due to naturally acquired immunity in addition to immunity from vaccination. Under reporting of coverage, potentially due to vaccines provided in the private sector, could suggest lower than true population immunity. In contrast, over reporting of vaccination coverage would provide a biased population immunity estimate that is higher than true immunity (Thompson, Odahowski, Goodson, Reef, & Perry, 2016). If a vaccine has a low seroconversion rate, vaccine immunogenicity is interfered by maternal immunity, or the vaccine loses potency from poor transportation or storage, the vaccination would not be effective, so serology would underestimate the proportion of children vaccinated.

A second option for inferring population immunity is to use disease surveillance data, which identifies immunity gaps in those who become diseased. Surveillance data for measles is

immediately notifiable and laboratory-verified; however the quality of this data can vary. Furthermore, surveillance systems suffer from selection bias. Only cases who present to the health system would be included, so there is often under reporting of cases (Plans-Rubio, 2014). Additionally, verification of cases is often done at the national level, so ensuring cases are reported from all geographic regions can be challenging for specimen transport. The age-distribution of cases allows for the identification of immunity gaps by age group. This often works best when there is a large outbreak of disease, covering a wide age range, so there is sufficient data to create an age-specific immunity profile. This could identify age-specific immunity gaps that existed before the outbreak and provide a sense of who was susceptible at the start of the outbreak. Deriving population immunity from surveillance systems can be challenging and could misrepresent immunity gaps.

Population immunity can be directly measured through seroepidemiology (Winter et al., 2018). Seroepidemiology can be defined as "the collection and use of data on the prevalence of antibodies in serum or related fluids to study the distribution and determinants of infection" (Cutts & Hanson, 2016). Monitoring the prevalence of these antibodies can be a powerful tool to design and monitor vaccination programs to maintain high population immunity; however, they are not often used because they are expensive and logistically challenging.

Potential uses for serological surveys

In order for a disease to be a strong candidate for seroepidemiology, there must be a good serological marker of protection, a valid test for measuring the antibody, and it must be a priority for the immunization program. Seroepidemiology can be used to measure population immunity in three different instances: prior to vaccine introduction, once a vaccine has been introduced

into routine immunization, and to assess the impact of a vaccination campaign. Within these broad scenarios, serological surveillance can have several different uses, though each would require different characteristics for each pathogen (Table 1). This could help not only assess where current population gaps are but how to target vaccination efforts to improve immunity levels (Trentini et al., 2017). Based on the granularity of available data, serosurveys could guide the age range or geographic areas where vaccination may be needed (Lessler, Metcalf, Cutts, & Grenfell, 2016).

Table 1. Potential uses for serological surveillance (Adapted from (Cutts & Hanson, 2016))

Potential use	Required characteristics
Assess vaccination coverage	• No natural infection or testing can distinguish antibody induced by vaccine from that by infection • Predictable immunogenicity • Antibody of known duration • Antibody levels correlate with number of doses of vaccine received
Estimate burden of disease	• Antigen or antibody correlates with infection
Select groups to target vaccination	• Protective antibody follows infection and is stable over time, so that seroprevalence by age signals age-specific or geographic immunity gaps
Determine elimination thresholds	• Protective antibody follows infection and is stable over time
Understand waning immunity	• Antibody is main correlate of protection
Predict risk of outbreaks	• Antibody is main correlate of protection • Protective antibody level is established
Estimate impact of campaigns	• Can account for antibody due to natural infection or immunization

The case for measles and rubella seroepidemiology

Measles is a highly infectious disease that was estimated to result in two million deaths worldwide each year before the availability of vaccine (Moss, 2012). For every case of measles, there are an estimated 12-18 secondary cases in a completely susceptible population. This is defined as the basic reproductive number, which determines the herd immunity threshold, and therefore vaccination coverage required to achieve herd immunity (Guerra et al., 2017). Measles vaccine began being used in the 1960s, and measles vaccine was included in the original Expanded Program on Immunization (EPI) which started in 1974 (Bland & Clements, 1998). In 2000, measles deaths accounted for 5% of under-five mortality (UNICEF, 2011). Following an increase in coverage with two doses of measles vaccine, estimated measles deaths dropped by 80% from 545,174 in 2000 to 109,638 in 2017 worldwide, with Africa accounting for 44% of measles deaths (Dabbagh et al., 2018).

Measles is a good candidate for serological surveillance because it induces a lasting antibody response and has established cutoffs indicating protective immunity. The correlate of protection for measles are reported to be measles-specificIgG antibody levels of >120 mIU/ml following vaccination when measured by plaque reduction neutralization assay (Chen, 1990). IgG levels are highest approximately 2 weeks after rash onset and are detectable for life (Gastanaduy, 2013). Laboratory testing for measles IgG is relatively straightforward and there are commercially available ELISA kits for blood and oral fluid samples which are relatively rapid and easy to use in the laboratory setting (World Health Organization, 2019). However, measles antibody detection cannot distinguish between natural infection and vaccine-induced immunity. Therefore, in a setting with circulating wild-type measles virus, serological surveillance would not be a good indicator of vaccination coverage, although it is still an

effective measurement of population immunity. This would more accurately identify the proportion of people susceptible than using vaccination coverage and measles case-based surveillance alone because it accounts for both natural immunity as well as vaccine induced immunity. Serological surveillance is currently being used in many European countries, the United States, Canada, and Australia and has been used for vaccine policy decision making (Nardone et al., 2008; Wilson, Deeks, Hatchette, & Crowcroft, 2012). Ten years of serological surveillance in the United Kingdom provided data on the susceptible population to be targeted for a national measles-rubella (MR) campaign in 1994, and the information for justifying the need for a second dose of measles-mumps-rubella (MMR) vaccine at 4 years of age (Osborne, Gay, Hesketh, Morgan-Capner, & Miller, 2000). Similarly, Canada's serological surveillance provided evidence for the introduction and timing of a second dose of measles-mumps-rubella vaccine to improve population level immunity (Ratnam, West, Gadag, Williams, & Oates, 1997).

Like measles, rubella is transmitted by the respiratory route. It is generally a mild disease in children but can cause severe sequelae in pregnant women, resulting in miscarriage or congenital rubella syndrome (CRS) in the fetus, a combination of conditions which include heart disease, blindness and deafness. For every case of rubella, there are an estimated 5-7 secondary cases in a completely susceptible population. Rubella vaccine began being used in 1969, and combination MR and MMR in the 1970s (World Health Organization, 2012). WHO published the first rubella vaccine position paper to guide countries in introducing rubella containing vaccine in 2000. The number of countries using rubella vaccine increased from 83 in 1996 to 130 in 2009 (Reef, Strebel, Dabbagh, Gacic-Dobo, & Cochi, 2011). Although not a major cause of childhood mortality, rubella is a major cause of congenital defects, estimated to affect 105,391 infants in 2010, with 37% of cases in Africa (Vynnycky et al., 2016).

Rubella is also a strong candidate for serological surveillance because rubella-specific IgG antibodies are a good serological marker of immunity, there is a valid test for measuring antibodies, and both infection and immunization result in lifelong immunity. The correlate of protection cutoff for IgG antibodies to rubella virus was established at 10–15 mIU/ml. (Plotkin, 2010). The limitation is that antibody detection cannot distinguish between natural immunity and vaccine-induced immunity. Serological surveillance provides more accurate data than disease surveillance reports because rubella is an under-notified disease due to the fact that rubella virus infection is subclinical in up to 50% of cases (Lanieri T, 2008). Even when there is no report of rubella cases, serological surveillance can provide evidence of circulating virus in the absence of vaccination (Clarke, Schild, Boustred, McGregor, & Williams, 1980). Therefore, serological surveillance is an important supplement to traditional case-based surveillance. The presence of IgG antibodies in serum can be used to indicate the immune status of a person whose history of rubella disease or vaccination is not known.

Given that both measles and rubella provide long-lasting immunity through vaccination or natural infection, including both in a serological survey is feasible. It is also efficient, as with sufficient volume, the same specimen can be used to test for antibodies to both measles and rubella viruses. Furthermore, including both measles and rubella together in a serosurvey is logical given their link in public health programs. The surveillance system for fever and rash illness captures cases of both, and laboratory testing is recommended for both pathogens to be done simultaneously (World Health Organization, 2019). Additionally, the combination measles-rubella vaccine being rolled out in many countries, may mean children susceptible to measles are also susceptible to rubella if they have not been vaccinated against either (Libman, Behr, Martel, & Ward, 2000).

Measles and rubella serosurveys

Measles and rubella serosurveys have been conducted for decades. One systematic review identified 477 measles and rubella serology studies through June 2014, mostly focused in developed countries (Thompson & Odahowski, 2016). The authors highlight the increased demand for serosurveys as burden of disease decreases to identify pockets of risk as countries move towards elimination. A recent review by the WHO Measles and Rubella Laboratory Network assessed 16 years of serological surveys, 68 for measles and 58 for rubella (Dimech & Mulders, 2016a). Of the 36 countries that conducted serological surveys, only 13 measles and 8 rubella serosurveys were conducted in developing countries, only five of which were in Africa. Most serosurveys were conducted for monitoring susceptibility through antibody detection as a cross-sectional survey (68%), though others were used to measure antibody persistence, estimate vaccination coverage and calculate vaccine immunogenicity. The study age ranges included infants, children, adolescents, adults, women of childbearing age. Study sizes varied from 70 to over 412,000, with around 30% of studies having over 2,000 individuals. The seroprevalence in these studies ranged from 60.8-95.9% for measles and 53.0-99.3% for rubella.

These serological studies varied in their methodologies and objectives. The specimen type ranged from serum to plasma and oral fluid to breast milk. Furthermore, some studies used enzyme immunoassays, while others used neutralization assays, automated immunoassays, hemagglutination inhibition assays and bioplex assays. In addition, these studies used a range of different quantitative cutoff values to classify seropositive results. The authors conclude that the results of different rubella and measles EIAs are not comparable despite being calibrated against WHO international standards. They also highlight the variability in tests and testing strategies and the implications that could have on seroprevalence. They recommend using only

serum/plasma specimens as a way of standardizing test results across serosurveys. Furthermore, they suggest calibrating the ELISA against plaque reduction neutralization tests (PRNT) using WHO international standards as another means to standardize testing between serosurveys, as PRNT has better sensitivity and was used to demonstrate correlation of protection for measles (Dimech & Mulders, 2016b).

A 2015 systematic review specific to rubella serology in African countries found that immunity ranged from 52.9 to 97.9%. Immunity among pregnant women ranged from 52.9% to 97.9% (Mirambo, Majigo, Aboud, Gross, & Mshana, 2015). Few studies compared rural and urban settings, but these did not find statistically significant differences (Amina et al., 2010; Barreto et al., 2006; Mwambe et al., 2014; Tahita et al., 2013). Almost all the studies were conducted among adults over the age of 15 years, and few studies provided any information on immunity by age groups (Amina et al., 2010; Kolawole, Anjorin, Adekanle, Kolawole, & Durowade, 2014; Kombich JJ, 2012; Mwambe et al., 2014; Onakewhor & Chiwuzie, 2011; Tahita et al., 2013). The authors also noted that seasonality in transmission was not included in any of the articles and that there is much information still missing to accurately characterize rubella dynamics in Africa (Mirambo et al., 2015).

Serosurvey guidelines have been developed to provide guidance for planning and implementing serosurveys for measles and rubella. The target audience is immunization program managers, epidemiologists and laboratorians working on measles and rubella elimination efforts. It outlines potential key stakeholders; survey planning and protocol development including design and methods; budget and timeline; ethical considerations; survey implementation such as sampling procedures, training and supervision of field and laboratory teams, data collection and management; laboratory methods including specimen transport and processing, choice of

specimen, serological assays and quality of control; and data analysis. As more countries introduce rubella containing vaccine and near measles and rubella elimination, serological surveys can serve as additional criteria to monitor the needed population immunity threshold for the certification of elimination.

Design and implementation of serosurveys

To plan for and implement serological surveys, there are several considerations to be made, as serological surveys are not appropriate to answer all public health research questions. The objectives and expected outcomes of the serosurvey need to be clearly outlined to guide the study design. Major methodological considerations to be considered include population sampling methods, selection of specimens to be collected, laboratory assays, and cost implications.

In terms of population sampling methods, serological surveys can use either previously collected specimens from biobanks for testing or prospectively collect new specimens. In both instances, samples can be acquired from convenience samples. Such convenience samples may include specimens collected for fever and rash illness surveillance, antenatal care specimens, human immunodeficiency virus (HIV) serological surveys, malaria indicator surveys, and other vaccine preventable disease (VPD) monitoring, such as Hepatitis B or Tetanus. It could also include samples collected in routine multi-purpose surveys such as DHS and MICS. Using convenience samples is logistically easier and can be cheaper due to the reduced cost of specimen collection but provides little control over sampling error and bias.

Prospective specimen collection allows for better control of data collection and sampling methodologies but can be costly and logistically challenging (Metcalf et al., 2016). Individual surveys for a specific antigen can be collected either as part of another activity, such as post-

vaccination coverage survey, or as a stand-alone serological survey for that specific antigen, and there are tradeoffs involved with doing each of these methodologies. Vaccination coverage surveys are often conducted at the household and could be combined with specimen collection to reduce fieldwork costs (Cutts et al., 2013). The WHO EPI cluster survey measures vaccination coverage based on caregiver recall and vaccination card but does not include biological specimen collection. Adding this component to vaccination coverage survey has been successfully done in low-income settings (Travassos et al., 2015). Advocates for collecting biospecimens as part of immunization coverage surveys have noted that there may be issues with logistical feasibility, cost implications and survey implementation (MacNeil et al., 2014). Other concerns are that the main survey may not have the same target population or sampling plan sufficient to meet the objectives of the serosurvey, and specimen collection might compromise the acceptability of the main survey (World Health Organization, 2015).

Method of specimen collection

There are different specimen types that can be used for serosurveys depending on the antigen and antibody class of interest: serum, dried blood spot, oral fluid, and breast milk. Serum and dried blood spots (DBS) are the most commonly used specimen types in serological surveillance and most commercially available measles and rubella assays are validated for serum. The concentration of antibodies in other sample types is generally lower than serum, which could decrease the sensitivity of the laboratory assay (World Health Organization, 2015). However, serum samples must be kept at cold temperatures, and specimens must arrive at the laboratory within 48 hours of collection, which can be logistically challenging. Therefore, an alternative is to place small volumes of dried blood on filter paper, as antibodies remain stable at room temperature for several days (Punnarugsa & Mungmee, 1991). Challenges with DBS are

that the eluted volume may be insufficient and require additional steps in the laboratory to elute and process specimens (Snijdewind et al., 2012). Concordance between serum and dried blood spots has been found to be as high as 92-93% for both measles and rubella IgG (Colson, Potter, et al., 2015; Helfand et al., 2001).

Dried blood spots from finger prick have been suggested for use in measles serological testing since as early as 1994 as an alternative specimen collection method that can provide similar serological results as serum (Parker & Cubitt, 1999). Finger pricks are a minimally invasive way to collect dried blood spots and can be collected through single-use lancet devices which minimize pain and bruising (Mei, Alexander, Adam, & Hannon, 2001). Finger pricks also have the added advantage of not requiring a phlebotomist or specialized healthcare professional, but rather can be performed by trained community health workers (Harvey et al., 2008; Novello et al., 1996). Additionally, finger pricks tend to require less time and have a lower risk for participants (World Health Organization, 2010).

Acceptability of serosurvey participation

Recruitment and retention of a representative population is crucial to the validity of research studies, including serosurveys (Durrheim, Orenstein, & Schluter, 2018). However, the factors associated with whether individuals choose to participate in a serosurvey are not well understood. The issue of selecting which specimen type to collect is tied to both laboratory assay selection as well as acceptability of specimen collection methodology. If people are more likely to accept finger prick than a vein puncture, this could change participation rates in the serosurvey. Collecting a biological sample could decrease response rates in a survey, and if more people refuse to provide a specimen, this could compromise the generalizability of the survey results (MacNeil et al., 2014).

The decision to participate in serosurvey research is affected by a balance of motivators and deterrents to participation (Almeida, Azevedo, Nunes, Vaz-da-Silva, & Soares-da-Silva, 2007). Motivating reasons for participation can include direct individual benefits such as financial incentives or perceived direct protection from disease (Dhalla & Poole, 2014). Perceived disadvantages for participating in a serosurvey include exposure to risks associated with taking blood samples, such as pain and crying among children. There are also concerns with the use of specimens for reasons other than those detailed in the study as well as not receiving results from the tests conducted (Chatio, Baiden, Achana, Oduro, & Akazili, 2016). However, beyond direct benefits, other reasons healthy individuals participate in research include altruistic reasons, community benefits, a desire to assist with overall disease reduction, and a feeling of accomplishment by contributing to research (Dhalla & Poole, 2014). Understanding the social benefits that motivate research participants provide an additional perspective to the direct personal benefits (Nyaoke et al., 2017). This belief in the societal benefits of research may be key to serosurvey participation, as serosurveys may not directly benefit the individual who is providing the blood specimen if they will not be given their test results.

Participants often consult other people, such as family, friends, or healthcare workers, before agreeing to take part in research; therefore, the decision making process is not just at the individual level (Almeida et al., 2007). Conceptual models, such as the socio-ecological model, have been used to identify different spheres of influence, like community-level influences, on research participation (Salihu, Wilson, King, Marty, & Whiteman, 2015). The socio-ecological model posits that people's behavior is determined not just by individual-level aspects but also by intrapersonal factors, interpersonal processes, institutional factors, community factors, and public policy (McLeroy, Bibeau, Steckler, & Glanz, 1988). Table 2 defines these levels and how they

could apply in the context of serosurveys. This interplay of factors can affect whether people participate in research, including the influence of social norms and community leaders (Nyaoke et al., 2017). The engagement of community and religious leaders through social mobilization has been recognized as an important component of community acceptance of survey participation. Additionally, using local health workers who are already known to the community to accompany teams and answer questions can help improve acceptability (Travassos et al., 2015).

Serosurveys may require additional resources for social mobilization because they collect biological specimens, often blood, from participants (Bryant Borders, Grobman, Amsden, Collins, & Holl, 2007). Refusal rates may be higher for surveys that collect blood specimens than surveys that collect only questionnaire data due to potential discomfort, added time for specimen collection, or participants not trusting what is being done with their blood (MacNeil et al., 2014). To monitor how non-participation could introduce bias, refusal rates for serosurvey participation are tracked and reported, as those who participate may be different than those who refuse. However, there are limited data explaining why individuals refuse to participate in serosurveys in sub-Saharan Africa (Travassos et al., 2015). Understanding the reasons behind acceptability of participation in serosurveys can help inform future serosurveys.

Table 2. Socio-ecological Model (adapted from (McLeroy et al., 1988))

Concept	Definition	Potential applications to serosurveys
Intrapersonal factors	Characteristics of the individual, such as knowledge, attitudes, behavior, self-concept	Knowledge about serosurveys (ex. blood collection, laboratory tests), attitudes towards participating in serosurveys
Interpersonal factors	Formal and informal social network and social support systems, including family, friendships	Family, community members opinions about participating in serosurveys (ex. Blood collection)
Institutional/organizational Factors	Institutions with organizational characteristics, and rules and regulations for operation	Institution/organization carrying out the serosurvey, and logistics of how serosurvey is being implemented
Community factors	Relationships among organizations, institutions and informal networks within defined boundaries	Relationships between organizations implementing the serosurvey
Public policy	Local and national laws and policies	Regulations around serosurveys

Costs associated with serological surveys

WHO estimates that a serosurvey could cost anywhere from $100,000 to over $1 million to implement (Patel, 2016). While much of this variability may be due to the design of the

serosurvey, understanding what the drives the costs of serosurveys beyond the required tools for specimen collection and laboratory processing is not well defined.

Some of the costs of serosurveys are costs that would be required for any survey, regardless of whether they include biospecimen collection or not, such as vaccination coverage surveys or DHS. These include data collection costs such as travel and fieldwork including participant enrollment and questionnaire administration (Luman, Worku, Berhane, Martin, & Cairns, 2007).

Another major driver of cost is the sample size of a survey, which is influenced by the prevalence of what is being estimated (Turner, Magnani, & Shuaib, 1996). Sampling strategies contribute to the cost of surveys differently, and understanding the implications of sampling on serosurveys could help determine their feasibility (Parker & Cubitt, 1999). Examining how much it costs to include an additional household versus an additional cluster in a serological survey could have implications on how decision makers' budget for serological surveys. Some serological surveys may choose to collect questionnaire data from a larger study population and collect biological specimens from a smaller subset of the study population. Understanding how different sampling strategies may contribute to the cost of serological surveys would help determine the feasibility of serological surveys.

Another potential means of reducing cost would be to refer participants to a health facility for blood collection rather than collecting blood in the community setting. However, requiring participants to reach the health care facility in order to participate can lead to selection bias, as this may introduce barriers for participants who would otherwise be willing to enroll. Collecting biological specimens at the households may overcome logistical barriers and increase

participation. However, collecting, processing and transporting blood specimens in the field can be expensive and logistically challenging to accomplish (Bryant Borders et al., 2007).

One of the reasons serosurveys can be costly is that biological specimen collection requires trained skilled workers or healthcare professionals for implementation. Additionally, biological specimens have an added cost of specimen collection supplies, storage and transport, laboratory testing, and additional human resources for laboratory processing (MacNeil et al., 2014). It is estimated that 60-70% of a serosurvey budget is laboratory-related supplies for blood collection, storage, transport, processing, and testing kits (World Health Organization, 2015). Collection and processing of DBS is thought to be cheaper than samples collected by venipuncture due to reduced costs of specimen collection and transport, though it requires an additional laboratory processing step which could offset the cost-savings in the field (Parker & Cubitt, 1999). Another way to reduce costs in the field would be to use already collected specimens, which could eliminate the costs of specimen collection.

While there have been concerns voiced about the cost of serosurveys, actual cost estimates are scarce. Most costing studies for measles and rubella have primarily focused on the cost-effectiveness of vaccine programs (Babigumira, Morgan, & Levin, 2013; Coppeta, Morucci, Pietroiusti, & Magrini, 2019). Pre-vaccination cost studies have assessed the costs of screening healthcare workers as well as antenatal screening of pregnant women (Ferson, Robertson, & Whybin, 1994; Lugner, Mollema, Ruijs, & Hahne, 2010). There have also been cost analyses of surveillance systems for vaccine preventable diseases (Duintjer Tebbens, Diop, Pallansch, Oberste, & Thompson, 2019; Erondu, Ferland, Haile, & Abimbola, 2019). A methodology was developed to cost integrated vaccine-preventable disease surveillance systems that includes calculating personnel costs and laboratory costs (Toscano et al., 2013).

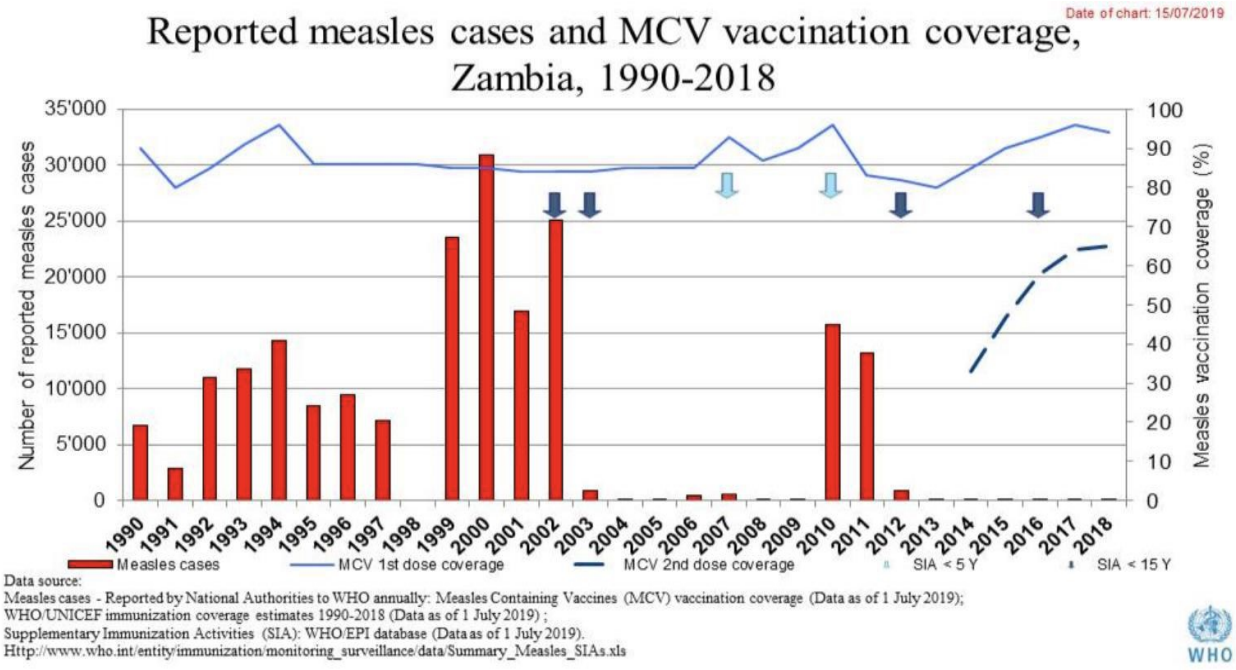
A comparison of vaccination coverage survey methodologies demonstrated that there was no difference in cost between conducting an EPI cluster survey and a systematic random sampling survey (Luman et al., 2007). Although the average time to survey a cluster, household and child differed between the two sampling methods; the total time to complete the first 30 households remained the same. Costing for time was broken down into time for travel, searching for a household to enroll, and interview. Another comparison between the EPI cluster survey methodology with probabilistic cluster survey methods noted that probabilistic methods provide a more statistically robust measurement with probability of selection for each cluster, household and child (Turner et al., 1996). However, because this methodology requires household listing of each cluster, it has higher costs than the EPI cluster survey.

Measles and Rubella in Zambia

Zambia is a low-income country in Southern Africa with a population of approximately 16 million and an annual birth cohort of 645,000 (GAVI, 2018). The country is divided into 10 provinces and 72 districts, with 41% of the population living in urban areas. Under-five mortality declined from 191 per 1,000 live births in 1990 to 64 per 1,000 in 2015, and is ranked 25th in the world (UNICEF, 2013). According to WHO estimated, the major causes of under-five mortality in Zambia in 2016 included neonatal disorders (33%), lower respiratory infections (14%), HIV (9%), diarrheal diseases (7%), and malaria and malnutrition (7%). Measles accounted for less than 1% of under-five deaths according to 2016 estimates (World Health Organization, 2018a).

Although the burden of measles in Zambia has been decreasing, it remains a periodic cause of mortality and morbidity (Figure 1). Prior to measles vaccine introduction in 1983, the disease affected mostly children. The first dose of measles vaccine is given at 9 months of age. Reported vaccination coverage increased from 74% in 1999 to 95% in 2005 (Centers for Disease Control and Prevention, 2005). From 2005-2016, WUENIC estimates of vaccination coverage ranged from 80-96%, which are generally lower than reported administrative coverage during this time (UNICEF, 2017). A second dose of measles vaccine was added to the EPI schedule in July 2013, and coverage climbed to 65% in 2018. In addition to routine measles vaccine, there have been periodic supplementary immunization activities conducted approximately every 2-4 years to address immunity gpas in children who may have missed routine immunization. These have ranged from being only a few districts to being nationwide campaigns, generally targeting 9-59-month olds or 9 month-14-year olds. These campaigns should have helped bolster population immunity to measles.

Figure 1. Measles cases and immunization campaigns in Zambia



Measles and rubella cases are captured through the fever and rash illness surveillance system in Zambia. All suspected cases are intended to be tested for measles and rubella IgM antibodies. Confirmed cases are those with positive laboratory testing, epidemiologically linked do not have laboratory confirmation but are linked to a confirmed case, and clinical cases are those that present clinically but may not have laboratory test confirmation. To assess the quality of the fever and rash illness surveillance system, several indicators are the surveillance system are routinely measured. The primary measles surveillance indicators are: (1) to meet a non-measles febrile rash illness rate of at least 2 per 100,000 population; and (2) to have at least 80% of districts report at least 1 suspected case of measles with a blood specimen per year. In 2012, Zambia met the first target; in 2013, it met neither of these targets; and in 2016, it met only the second (Masresha et al., 2017; Masresha et al., 2014). Although the surveillance system is imperfect, periodic outbreaks of both measles and rubella have been documented (Table 3).

Table 3. Cases of measles and rubella in Zambia reported to WHO, 2010-2016[*]

	2010	2011	2012	2013	2014	2015	2016	2017	2018
MEASLES	15,754	13,234	896	35	9	9	8	13	11
RUBELLA	13	1,124	134	183	74	94	34	16	2
CONGENITAL RUBELLA SYNDROME	0	0	0	0	10	3	0	0	-

NOTES: Source is WHO - reported incidence by country – Zambia.
[*]Total confirmed cases reported. These include all cases confirmed by laboratory testing, or epidemiological linkage, plus those clinical cases that were reported without a laboratory specimen or epidemiologic linkage.

Currently there are no national goals for measles, rubella or CRS control or elimination in Zambia, although there is a WHO AFRO regional measles elimination goal by 2020. Rubella is not a reportable disease in Zambia and is therefore primarily detected through laboratory testing of suspected fever and rash cases. According to the surveillance system, peaks of rubella occurred in 2004, 2005, 2008 and 2009, when measles positivity was low. There was a large outbreak in 2011, which occurred simultaneously with a large measles outbreak. Rubella appears to have a seasonal pattern in Zambia, with most cases occurring between August and December. It was estimated that children ages 5-14 years represented most rubella infections (M. Mazaba et al., 2012). Zambia does not have sentinel surveillance for monitoring CRS. In 2012-13, case reports documented the first laboratory-confirmed CRS cases in Zambia, all from the same hospital, though these are not counted in the WHO reports of CRS (M. L. Mazaba et al., 2014). One mathematical model predicted the incidence of CRS to be 123 per 100,000 live births (95% confidence interval 29-246) (Cutts & Vynnycky, 1999). Given that the yearly birth cohort is estimated at 644,972, the number of CRS cases would be expected to be much higher than the reported number of 10 in 2014, indicating an underreporting of CRS cases (GAVI, 2018). An update of this model that included a systematic review estimated that Zambia had over 1,000

cases of CRS born per year (Vynnycky et al., 2016). In 2016, Zambia utilized GAVI funding to introduce rubella containing vaccine through a supplementary immunization campaign that covered 9 month-14-year olds, and subsequently replaced measles only vaccine in the routine immunization program.

A few serological surveillance studies have been conducted in Zambia prior to rubella vaccine introduction. In 1979, a serological surveillance study found 75% immunity to rubella by the age of 13 (Watts, 1983). More than 30 years later, another rubella serological survey tested donor blood for rubella IgG and found 91.9% seroprevalence among women older than 16 years of age (Mazaba-Liwewe ML, 2015). This study found no association with sociodemographic factors such as economic status, education, marital status or urban/rural residence. The authors note that although they did not find an association with age, this could have been due to the small sample size of 124 women (M. L. Mazaba, Monze, Babaniyi, Siziya, & Michelo, 2015).

In 2016, a retrospective serological survey used specimens of children 5-15 years old, collected for HIV and malaria studies and tested them for measles and rubella IgG. The objective was to compare the seropositivity of HIV uninfected children with HIV infected children, both on and naïve to antiretroviral therapy. This study found that a higher proportion of HIV-uninfected children (92.5%) were seropositive for measles than HIV infected treatment naïve children (74.1%) and HIV infected on ART (71.9%). For rubella, 54.7% of HIV uninfected children were seropositive, compared to 41.7% HIV infected treatment naïve and 49.6% HIV infected on ART. For rubella, seropositivity significantly increased with age, suggesting that population immunity reaches over 80% by the age of 15 years (Sutcliffe et al., 2017).

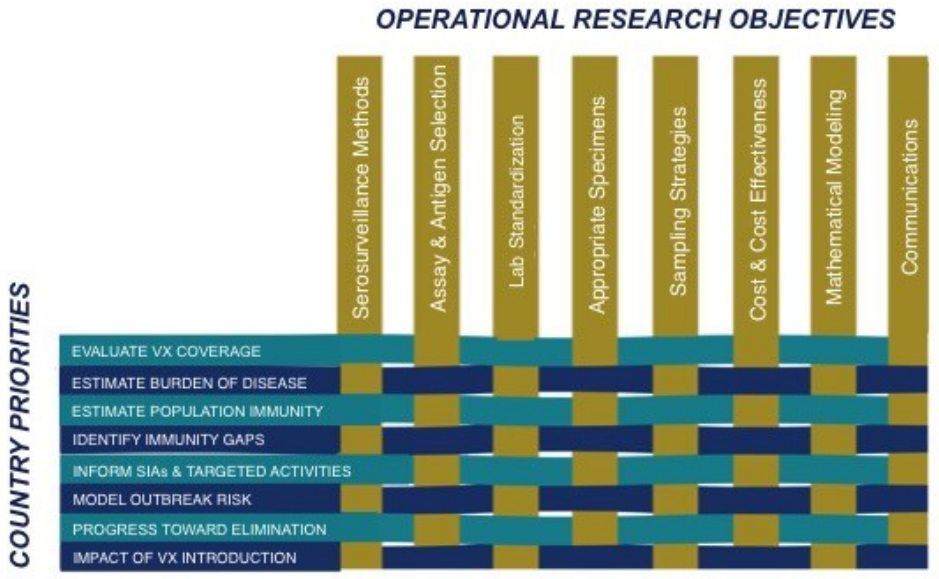
The results of these serological surveys in Zambia are in line with other African serological surveys which found 84-94% seropositivity to rubella among women of reproductive

age (Goodson et al., 2011). However, most of them used a small study population that was not nationally representative or covered narrow age groups. Little is known about the full age-range susceptibility profile of rubella in Zambia. Additionally, previous serological surveys used only biospecimen repositories.

Parent study: Strengthening Immunization Systems through Surveillance (SISS)

To address some of the methodological questions for serological surveys, the International Vaccine Access Center developed a project funded by the Bill and Melinda Gates Foundation to conduct research studies on these issues. The overall goal of the project is to assess the feasibility and utility of using serological data to monitor and guide immunization programs in low income countries. This includes addressing epidemiological, technical and operational issues to demonstrate the capacity to generate valid and timely serological data to guide routine, supplemental and targeted immunization activities. To accomplish this, SISS established demonstration projects in India and Zambia. In both field sites, research activities aim to answer operational research objectives for serological surveys that are generalizable, while taking into account specific country priorities (Figure 2).

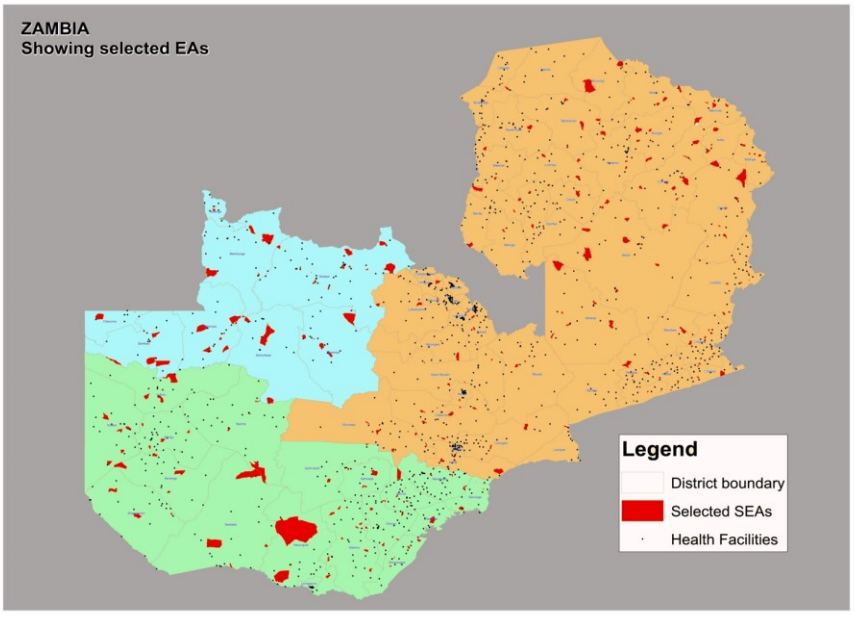
Figure 2. SISS intersection of country priorities and operational research objectives



First serosurvey: ZamPHIA

In early 2016, the Zambia Population HIV Impact Assessment (ZamPHIA) was conducted as a cross-sectional community survey to estimate HIV incidence and prevalence. This study collected survey data and venous blood specimens from adults 15 to 49 years of age in every household selected and children younger than 15 years of age in every other household, with dried blood spots collected from children under 2 years of age (Ministry of Health, Zambia, 2017). This study was powered to estimate provincial HIV incidence by selecting enumeration areas in each province (Figure 3). This study reported 11% of households refused to provide blood specimen and only 68% of eligible children participated (Columbia University, 2016). After HIV testing was completed, residual plasma and dried blood spots from this study were frozen in a biorepository at -70°C at the Tropical Disease Research Center in Ndola, Zambia. Residual plasma and DBS samples from this study were tested for anti-measles virus and anti-rubella virus IgG antibodies.

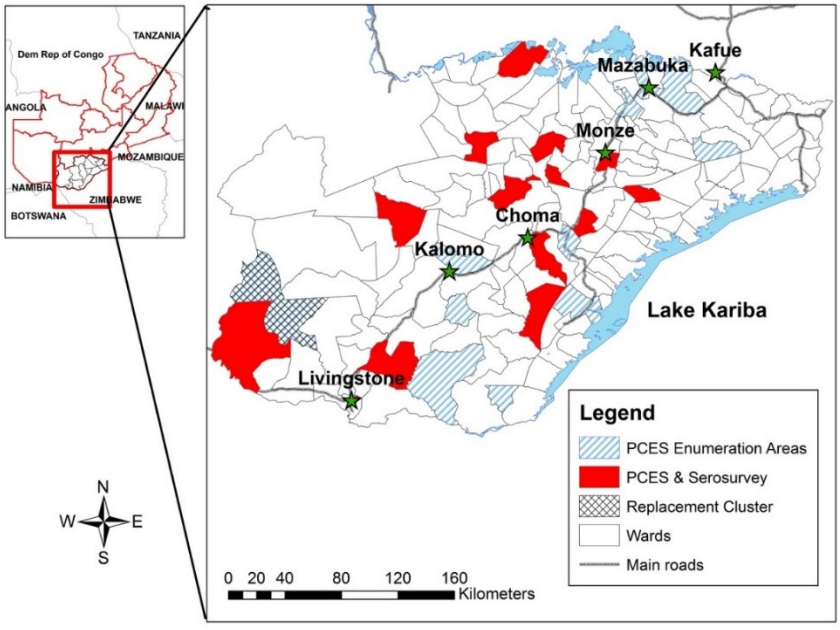
Figure 3. Selected enumeration areas included in ZamPHIA study



Second serosurvey: nested within PCES

The second serosurvey to assess measles and rubella immunity was conducted as part of a post-campaign vaccination coverage survey conducted by the Ministry of Health in Southern Province, Zambia. Southern Province was selected based on administrative immunization coverage and the capacity of field and laboratory teams at Macha Research Trust, an existing field site established in 2003 that had been working on malaria and HIV research. The nested serological survey used the same sampling strategy as the post-campaign vaccination coverage survey. Twenty-six clusters were selected in Southern Province (Figure 4). Within these, households with children in the post-campaign vaccination coverage survey target age group (9 months to less than 15 years) were eligible for enrollment in the nested serological survey. Dried blood spots were collected from participants 9 months of age and older to test for anti-measles virus and anti-rubella virus IgG antibodies.

Figure 4. Selected enumeration areas included in the post-campaign vaccination coverage survey



Introduction to dissertation

In the following chapters, I present an introduction, methodology, results, discussion and conclusions separately for each of the three dissertation aims. Finally, a concluding section pulls together all the information and provides public health significance and next steps for future research. All papers are embedded in the parent study of strengthening immunization systems in Southern Province, Zambia.

Specific aims

1. **Characterize factors that impact participation in serosurveys:** This aim documented the barriers and facilitators to participating in community-based serological surveillance during the post-campaign coverage survey of measles and rubella.
2. **Cost of serosurveys:** For this aim, we developed a costing framework for serological surveillance and estimated the costs of collecting serological specimens during a vaccination coverage survey to estimate population immunity levels for measles and rubella.
3. **Change in seroprevalence following a vaccination campaign:** To assess the change in seroprevalence of measles and rubella pre and post-vaccination campaign from two serosurveys, we adjusted for different survey design methodologies.

Aim 1 documents the factors associated with participation in serosurveys from the perspectives of data collectors who participated in the nested serosurvey and caregivers who were in communities eligible to participate in serosurveys. We used the socioecological model as

a framework to characterize barriers and facilitators to serosurvey participation. Aim 2 adapts a framework from integrated disease surveillance to capture the costs of doing a serosurvey nested in a post-vaccination campaign survey. We captured costs by both input and serosurvey function to evaluate which factors contribute the most to the cost of serosurveys. We also vary the sample size parameters to estimate the effect on the cost of serosurveys.

Aim 3 uses two serosurveys conducted with different methodologies to estimate the change in seroprevalence before and after a measles-rubella vaccination campaign. We explore the adjustments and factors to consider when comparing two serosurveys that used different data collection and laboratory methodologies. Finally, we present the implications of serosurvey design and considerations for implementing future serosurveys. Each chapter addresses a different aspect of the feasibility of using serosurveys to monitor population immunity, and the discussion chapter ties together the implications for serosurveys.

Data sources

This thesis dissertation uses information from two different serosurveys: (1) ZamPHIA, a serosurvey that used a biorepository from specimens collected for an HIV study nationwide in the first half of 2016, and (2) a serosurvey conducted in November-December 2016 simultaneously with a post-campaign vaccination coverage evaluation survey (PCES) in southern province. The first study includes specimens collected before Zambia conducted its nationwide measles-rubella vaccination campaign and introduced rubella containing vaccine; whereas the second serosurvey contains specimens that were collected after the vaccination campaign.

Table 4. Data sources used for each paper

	Paper 1: Acceptability	Paper 2: Cost	Paper 3: Pre-post
A) ZamPHIA			
Quantitative epidemiologic data			■
Laboratory data			■
B) Post-campaign coverage survey (PCES)			
Quantitative epidemiologic data		■	■
Laboratory data		■	■
Budget information		■	
Qualitative data	■		
C) Post-introduction evaluation for measles-rubella vaccine			
Qualitative data	■		

Aim 1 uses qualitative data collected in two different phases. For the first phase we conducted focus group discussions and interviews with data collectors who participated in the PCES serosurvey. The second phase surveyed caregivers at health facilities who were present at the time of the post-introduction evaluation for measles-rubella vaccine in areas that were eligible to participate in serosurveys. They were interviewed about barriers and facilitators to serosurvey participation for both themselves and their families. Aim 2 uses the PCES serosurvey to estimate the financial costs of a serosurvey using an ingredients-based approach. Data sources included the serosurvey budget and program records and interviewing administrative personnel. Aim 3 analyzes laboratory results and epidemiological data from Southern Province in the two serosurveys: ZamPHIA and the PCES.

Ethical approvals

The ZamPHIA study was originally approved by the Zambian Ministry of Health. Testing of the biorepository was approved by the Johns Hopkins School of Public Health IRB as well as Zambian National Regulatory Authority. Written consent was provided by all participants for the original HIV testing, as well as whether they would allow their specimens to be stored for future testing. The post-campaign coverage survey received ethical approvals from the Zambian Ministry of Health, as well as IRB approval at Johns Hopkins School of Public Health and Macha Research Trust in Zambia and was approved by the Zambian National Regulatory Authority. Written informed consent and/or assent was obtained by all participants, as age appropriate. The post-introduction evaluation was approved by the Zambian Ministry of Health as part of programmatic activities. Oral consent was provided by participants.

Paper 1- Acceptability of a serosurvey for vaccine preventable diseases in rural southern Zambia: surveyor and community perspectives

Abstract

Background: The factors associated with whether individuals choose to participate in survey research are not well understood. Serosurveys may require additional resources to ensure participation because they collect biological specimens, often blood, from participants. Understanding perceptions from multiple perspectives, including the perspectives of both data collectors and participants through the socio-ecological model contextualizes individual, interpersonal, and structural level influences on survey research participation.

Methods: We used a mixed methods approach to characterize reasons for serosurvey participation in communities in Southern Province, Zambia where a serosurvey was conducted in 2016. For the first phase, we conducted focus group discussions and interviews with 24 data collectors who participated in a measles-rubella serosurvey in 2016. The second phase surveyed 34 caregivers at health facilities to identify barriers and facilitators to serosurvey participation. Emergent themes from the data were then classified into a socio-ecological model using individual, interpersonal, and structural level constructs.

Results: Common themes emerged from the data collectors as well as the caregivers surveyed. At the individual level, providing incentives was a motivator, and religion was described as a barrier to serosurvey participation. At the interpersonal level, family dynamics and community influences could help or hinder serosurvey participation; however, community health workers

were consistently named as facilitators of participation. At the structural level, concerns about specimen collection, who was selected to participate, and not receiving test results arose as potential barriers. The most frequent facilitator was provision of information about the purpose of the serosurvey (85%). The most frequent barrier was being unsure of what is being done with their blood (53%). For specimen collection type, caregivers preferred finger prick blood collection over both venous blood draw (59%) and oral swabs (71%).

Discussion: Interpersonal relationships and structural issues related to serosurvey implementation were the key motivators for participation in a serosurvey. Provision of information ahead of the serosurvey to answer questions related to blood collection and communicating through a trusted vehicle such as the community health worker were particularly important to all participants.

Keywords: acceptability, serosurvey, specimen collection

Background

Recruitment and retention of a representative population is crucial to the validity of research studies, including population surveys. However, the factors associated with whether individuals choose to participate in research are not well understood. For some studies, such as clinical trials, reasons for participation can include direct individual benefits (Chatio et al., 2016). However, beyond direct benefits, other reasons healthy individuals participate in clinical trials include altruistic reasons, community benefits, and a feeling of accomplishment by contributing to research (Dhalla & Poole, 2014). This belief in the societal benefits of research is also applicable in the context of survey research, whether there are individual or community level benefits.

Conceptual models, such as the socio-ecological model, have been used to identify influences, such community-level influences, on research participation (Salihu et al., 2015). The socio-ecological model posits that people's behavior is determined not just by individual-level aspects but also by intrapersonal factors, interpersonal processes, institutional factors, community factors, and public policy (McLeroy et al., 1988). This interplay of factors can affect whether people participate in research, including the influence of social norms and community leaders (Nyaoke et al., 2017). The engagement of community and religious leaders through social mobilization has been recognized as an important component of community acceptance of survey participation (Travassos et al., 2015).

Serosurveys may require additional resources for social mobilization because they collect biological specimens, often blood, from participants (Bryant Borders et al., 2007). Refusal rates may be higher for surveys that collect blood specimens than surveys that collect only questionnaire data due to potential discomfort, added time for specimen collection, or participants not trusting what is being done with their blood (MacNeil et al., 2014). To monitor how

non-participation could introduce bias, refusal rates for serosurvey participation are tracked and reported, as those who participate may be different than those who refuse. However, there are limited data explaining why individuals refuse to participate in serosurveys in sub-Saharan Africa (Travassos et al., 2015).

To improve participation in serosurveys, better understanding is needed on how communities perceive serosurveys. Understanding perceptions from multiple perspectives, including the perspectives of both data collectors and participants, can provide a more comprehensive view on what influences participation. We conducted a qualitative analysis to characterize reasons for serosurvey participation in communities in Southern Province, Zambia where a serosurvey was conducted in 2016 following a measles-rubella vaccination campaign.

Methods

Study setting

The study was conducted in Southern Province, Zambia, the third most populous province in Zambia with 1.6 million inhabitants (Zambia Central Statistical Office, 2012). The province is primarily agrarian, with a malaria prevalence of less than 1%, an estimated HIV prevalence of 13% among adults, and first-dose measles vaccination coverage of 86% (Zambia Central Statistical Office, 2014). The infant mortality rate is 44, compared to a national average of 45 (Zambia Central Statistical Office, 2014). There were at least two serosurveys conducted in different parts of Southern Province in 2016. In early 2016, an HIV incidence and prevalence survey, ZamPHIA, was conducted by collecting blood in a community setting; 11% of individuals participated in the survey but refused blood collection (Columbia University, 2016). In mid-2016, a serosurvey to assess measles and rubella immunity was conducted among

communities in this area as part of a vaccination coverage survey conducted by the Ministry of Health. This serosurvey collected dried blood spots from participants who provided written consent after being informed of the purpose of the study (Hayford et al., 2019). This serosurvey also included a sensitization plan to inform key stakeholders the purpose and procedures of the serosurvey. During the serosurvey, data collectors were assigned community health workers from the clinics who they were intended to coordinate with during the serosurvey. Despite these efforts to ensure participation, 12% of individuals at enrolled households refused to participate in the serosurvey (Mutembo et al., 2018).

Study Design

We used a convergent mixed-methods design where qualitative and quantitative data were collected sequentially, analyzed simultaneously, and interpreted together. The study was conducted in two different phases in Southern Province, Zambia. The first phase was qualitative and was conducted immediately following the measles-rubella serosurvey in December 2016. We conducted in-depth interviews and focus group discussions with data collectors to evaluate the implementation of the serosurvey and their perception of community acceptability. The second phase was conducted in April 2017 among caregivers at health facilities in districts that were eligible to participate in the serosurveys. The cross-sectional survey consisted of questions related to what would motivate or prevent them from participating in a serosurvey.

Participants and Data Collection

In the first phase, the 36 data collectors who were part of the serosurvey teams were purposefully sampled to include representation of at least one member from each team that

participated in the serosurvey. From the personnel list, team supervisors were prioritized, and then data collectors who were involved in blood collection. Participants were recruited during the serosurvey and invited to participate immediately after data collection was completed. We aimed to include participants that had different roles on the team, and we stopped once data saturation was reached. Interviews and focus group discussion (FGD) guides included questions on how the data collectors were received by the community and perceptions of community acceptability of serosurveys. Interviews were conducted individually with supervisors so as to not influence non-supervisors, and FGDs were conducted with non-supervisory data collectors in groups of 6-8 individuals, with a local partner who specializes in qualitative research and was external to the serosurvey, to help facilitate. We obtained verbal consent from all participants. All interviews and FGDs were conducted in a private room and were done in English, audio recorded, and transcribed. Reflexive memos were written following each interview and FGD, as well as throughout the data analysis process.

For the second phase, a convenience sample of caregivers attending health facilities were selected. During a two-week period, caregivers who were present at the health facility at the time of the supervisory visit were recruited by a healthcare worker in the waiting room. Participants were not required to have participated in a serosurvey previously, but facilities were in areas which were eligible to participate in the previous serosurveys. It was reiterated during the oral consent process that the questionnaire would have no bearing on the care they received. The caregivers were asked questions related to the acceptability of blood collection in the community, including previous serosurvey participation, barriers and facilitators to serosurvey participation, specimen preference, and self-efficacy, the belief in one's ability to make one's own decisions. We used results from the first phase to develop the survey questions for the

second phase. Questionnaires were translated into Tonga and back translated. Questionnaires were administered in Tonga by a trained local collaborator in a private area in the waiting room of the health facility. All data were entered in a tablet and uploaded to a secure cloud server daily. Debriefing meetings were held after questionnaires were completed at each health facility to review data quality.

Ethical and regulatory approval was obtained from the institutional review boards at Johns Hopkins University School of Public Health and Macha Research Trust, and the study was approved by the Zambia National Health Research Authority.

Data Analysis

Qualitative data from the first phase were analyzed using a grounded theory approach in Atlas TI (version 10.1). Grounded theory allows the data to speak for itself without using a priori assumptions (Glaser, 1967). There were several rounds of codebook development as well as coding, including initial open coding to generate categories, followed by axial coding to relate these categories, and finally grouping by theory (Charmaz, 2006). Coding discrepancies between the two analysts were discussed until a resolution was reached. The constant comparison approach was used to generate themes through simultaneous coding and analysis (Glaser, 1967). Preliminary analysis of the themes that arose during the qualitative analysis of the first phase informed the development of the survey questionnaire for caregivers in the second phase.

Quantitative data from the second phase were cleaned and analyzed using STATA 14. Descriptive statistics were performed to look at frequencies, and grounded theory was used to code the answers of open-ended questions in Excel. Quantitative and qualitative data were

initially cleaned separately and then analyzed together, classifying emergent themes into a socio-ecological model as a framework using individual, interpersonal, and structural level constructs.

Results

Participant characterization

Two focus groups were conducted with 13 data collectors, and 11 semi-structured in-depth interviews with supervisors of the serosurvey teams were conducted (Table 5). Thirty-four caregivers were surveyed from 9 different health centers in 3 districts in Southern Province, Zambia (Table 6). These districts had also participated in the post-campaign coverage survey and serosurvey that took place in 2016. All participants were female. Most of the mothers were younger than 30 years of age (68%). The most prominent religion was Seventh Day Adventists (38%), which does not permit blood collection. Almost half of those surveyed reported having traveled for longer than one hour to arrive at the health facility. Most of the caregivers were at the facility to bring their children to the vaccination clinic, most of whom were under five-years of age (91%).

Ten caregivers (29%) reported having participated in a household-based survey in the past. These included surveys for different health purposes including malaria, HIV, and sanitation. Of those, eight caregivers (80%) reported that the surveys collected blood or saliva, about half (50%) received their test results, and three caregivers (30%) had taken part in more than one household survey. When asked directly if they would allow finger prick blood collection at their households, 74% accepted for themselves, and 62% said they would allow their children to participate.

Emerging Themes

Results were categorized by acceptability factors that affect participation in serosurveys, including barriers and facilitators at each socio-ecological model level: individual, interpersonal, and structural. Individual influences included personal motivation such as incentives and religious beliefs. Interpersonal influences affecting participation in serosurvey decision making included community, family structure, and the community health worker influence on participants. Finally, structural influences included factors associated with survey implementation. These included questions related to specimen collection, including specimen preference type, who is asked to participate in serosurveys, and provision of test results. These acceptability themes are summarized by socio-ecological model level in Figure 5 and presented below.

Acceptability: Individual Influences

According to survey results, there were individual motivators classified as facilitators that encourage serosurvey participation including incentives, altruistic notions, and the personal desire to know more about health issues (Table 7). Similarly, data collectors noted participants wanted compensation for their participation. Religious beliefs were mentioned as a barrier to specimen collection by both the caregivers and the data collectors.

Incentives

Data collectors identified compensation as a specific individual level incentive. One data collector remarked:

We discussed (incentives) in workshop. That it would look as if we were paying for blood, but people really wanted to be compensated for their blood.

Data collectors noted that even if payment could not be made so that participation would not be perceived as coercive, compensation of some form was asked for by participants, as evidenced by this data collector:

On the same collecting blood, others were saying, 'you should compensate us'. Simple stuff like a drink, milk. That's what normally happens when others go out to collect blood. So why are you not compensating us?

Religion

Religious beliefs were primarily described as a barrier to serosurvey participation due to blood collection, as mentioned by 15 caregivers (44%). Zionism, a religious sect that does not believe in Western medicine ("Zionism," 2019), and Seventh Day Adventists were mentioned as religions that did not allow provision of blood specimens. Two caregivers also mentioned satanism, the worship of Satan or obsession with evil, as a barrier to blood collection. Many of the concerns that data collectors reported related to traditional beliefs that blood was used for witchcraft and satanic rituals, as articulated by this data collector:

You know, the people, they think that when you get blood from them, maybe you want to do rituals on them. That's what affected mostly the blood collection. And there are some areas where they believe really too much into satanism.

Data collectors reported being told that they were not welcome to collect blood because community members held the belief that data collectors themselves were Satanists.

When you start explaining to them [why we are here], they get surprised and some were even chasing us, telling us, "Go!", "Why do you want our blood? You are just Satanists!"

Despite reports of the community calling data collectors Satanists, a supervisor pointed out she did not believe there was a prominent satanic community in the areas where the serosurvey occurred.

The belief around here was, they were like, maybe you want to…. offer my blood as a sacrifice. Maybe you are Satanists. Yes. They believe in Satanists collecting blood from people.

Facilitator: Does that happen a lot here?

They've never even seen it. Neither have I. So [satanism] is just rumors.

Acceptability: Interpersonal Influences

The decision of whether or not to participate in a serosurvey falls under a broad healthcare decision making umbrella. Survey participants identified several interpersonal influences on healthcare decision making, including themselves, family members, and community health workers (Table 7). Data collectors similarly mentioned family members and community health workers as interpersonal influencers, but also added the belief that community norms and specific community members influenced individual choice to participate. With regards to interpersonal influences, both caregivers and data collectors noted the importance of providing information about the objective of the serosurvey and having this come from someone the community trusts, such as the community health worker.

Family

Family dynamics played a role in potential serosurvey participation, as noted by 14 caregivers (41%) who cited fathers or grandparents as influencing their healthcare decision making process. Data collectors also noted that permission from caregivers to allow children to participate in the serosurvey was challenging in some areas because mothers required permission from the fathers, or even tried to have their children participate but the fathers refused by even hiding children, as told by this data collector:

> *The husband said, "no, I cannot do this." The wife… wanted to participate, but the husband got one of the children [and took] him in the house. Again, [he] called another [child], and sent to get another [child]. The mother was like, "what do I do now?"*

In addition to the need for approval from the child's father, data collectors stated that women also needed to be present for a child to participate in the serosurvey. This is because mothers have the information to answer the questions regarding vaccination history, as noted by this data collector:

> *I guess that made me understand that the men that head the households don't really have enough information on their children and their children's health.*

Additionally, older children, even young adults, tended to default to the decision of their parents as to whether they participated in the serosurvey, as this data collector relayed:

> *Generally, if the parents refused…even if the kids [were] 15-16 [years old]…they would say dad doesn't want [them to participate]. We'd find maybe even 18-20 [years old], they would accept because we told them what the survey was about, but if daddy says none of my children, they would also back out. They were adults, but they start off saying no because parents said no, even if they want [to participate].*

Community

Data collectors highlighted the influence of the community on serosurvey participation. For areas where there were refusals, it seemed there had either been poor social mobilization efforts or community sensitization was insufficient. One data collector relayed to us the thought process of participants in a community where she believed they had gathered to make a collective decision on participation in the survey:

> *For example, you visit me at this house. You also visit the other house. If I'm not clear, I'll go and ask my neighbor, 'did you hear what these people say?' And sometimes the community will want to meet. 'Let's do this when they come, let us all refuse.' By the time you go, you try here, you try there. Like one village where we went, everyone refused. And we definitely knew these people could have had a meeting after we passed yesterday, they met.*

Some data collectors reported that communities said they could not participate because the village headmen and local health facilities had not been informed of the survey, indicating community leadership served as gatekeepers to serosurvey acceptance:

> *Their concerns were…if it's from the Ministry of Health, they always pass through the clinics, or the health centers/facilities to say or to announce to the community to say if you come on such and such dates to the clinic, there will be this. Yes, they know. So the villages, the headmen, most of them, they were not aware. So they were like, we cannot participate because the headman does not know. So they were all referring to the headman.*

Community health worker

Another influence in the acceptability of serosurvey participation was the community health worker (CHW). Among caregivers, one-third had received health information from community health workers, including information about vaccination. CHWs were viewed as important facilitators by both data collectors and caregivers for two primary reasons: they were recognizable by the community, and they were a source of trusted information.

Caregivers articulated that it was important to have someone they recognized on the survey team, as this was an important factor in deciding whether or not to participate in a serosurvey:

> *The people doing the surveys must go through the health center and come with people from the health center. Then we know they are real. I would not accept unless you come with someone I know from the health center.*

This sentiment of having someone the community trusts was named as a facilitator to serosurvey participation by one-third of caregivers.

Overwhelmingly, data collectors agreed that having a CHW accompany the serosurvey teams was helpful for both knowing where to go in the community as well as sensitization of the community. One data collector noted:

> *At the clinic [we picked up] the health worker, and... the community health worker. We used to move with the two of them. [When] we would go to certain households, [the community health worker] was the one who first, started introducing us, so it was a bit easy.*

Almost all caregivers (85%) reported that the provision of information about the purpose of the serosurvey was the primary facilitator to encourage participation. This sentiment was echoed by

data collectors, who recommended sensitization in the communities ahead of time, as mentioned by this data collector:

> *Because most of the people in rural areas are illiterate, so it's very difficult for them to understand when you go there to explain something. Even if you explain something in depth, they will keep on asking you the same question. So if they visit them, they explain…maybe it can be easier when other people come in now to do surveys in their areas.*

Data collectors believed that providing information in advance about the serosurvey to the communities facilitated implementation in some areas, as noted by another data collector:

> *[For] sensitization I think it's also important to give the correct information to people who are requested to go in the community to sensitize. It would make the work very easy.*

Community health workers should be the ones to provide the information about the purpose of the serosurveys to improve participation, as highlighted by this data collector:

> *I think the best thing to do is, those places...if they can make use of the CHWs to sensitize them. So that when there's such a program, the people are aware, and they know the reason why people travel from distant places to go to their places to do such programs. Because the level of understanding is what is lacking.*

<u>Acceptability: Structural influences</u>

Structural issues were among the most common themes. Among data collectors, structural level influences on participation were related to specimen collection, including amount of blood and where blood was being taken; who was selected to participate and who was excluded; and desire for test results. In the caregiver survey, being unsure of what was being

done with the blood and fear of test results arose as significant barriers to participation (Table 7). Both the qualitative and survey data indicated that receiving test results was an important facilitator to serosurvey participation. While questions about selection criteria for participation did not arise in the survey data, caregivers did mention that multiple researchers working in the area was a barrier. Both qualitative and survey results specifically referenced HIV testing as a concern for serosurvey participation; however, data collectors also noted that some participants desired HIV testing. Structural level barriers and facilitators were grouped into issues related to specimen collection, selection criteria for participation, and the results of testing.

Specimen Collection

Barriers named by both caregivers and data collectors were primarily centered on issues with specimen collection. Most caregivers (53%) listed lack of trust in what was being done with the blood as the primary barrier to participation. Similarly, data collectors mentioned that potential participants asked many questions despite being explained during the consent process: "*Why are you taking our blood? Why are you studying this?"* There were also some questions related to rubella virus, the vaccines, and how antibodies worked.

In the qualitative data, data collectors noted comments about specimen collection related to past experiences with serosurveys. In areas where the community had participated in an HIV serosurvey earlier that year, some of the questions that arose were related to their experience with that survey. One supervisor noted the amount of blood as a concern.

> *Before consenting, they really wanted to know how much blood we were collecting. They were referring to previous survey…They were saying ZamPHIA collected a lot of blood, so how much are you collecting from us.*

The primary concern that data collectors noted from the community was "*Where are you taking our blood?"* As one supervisor highlights, this concern refers to a previous serosurvey:

> *In general, they were just asking, where the blood is being taken. Because…the last survey which happened for ZamPHIA, they were collecting about 14 mL of blood, which they were using to test HIV, syphilis and hepatitis B. So, people were actually concerned about that one. So when you just mention blood, they would be like, where do you take our blood?.... They would ask a lot of questions over the blood that was taken.*

In terms of where specimen collection should occur, the survey found that half of caregivers thought having people come to a central location, such as a clinic within the community, would facilitate serosurvey participation.

Selection criteria

In terms of who is selected to participate in serosurveys, both caregivers and data collectors mentioned concerns about the same communities being repeatedly asked to participate. One caregiver listed too many researchers coming to collect blood from the community as a barrier. This sentiment was echoed by the data collectors, as noted by one:

> *Mostly people go there to collect blood. Different programs, from MOH [Ministry of Health]. So every time it's just blood, blood, blood...*

In the qualitative data, data collectors noted additional concerns from the community as to why certain people were being included in the survey and others excluded. In some cases, this was from participants who were selected for the serosurvey, asking why everyone was not being included. In other cases, it was community members wanting to participate, and asking why they were being excluded. Both experiences are mentioned by this supervisor:

You would find that the household that you…pick. Those that were picked will say, "why us? Why are you not getting everyone?" So sometimes you would find that those that are left out, like, "why have you left us?" There was someone [who] got upset. "We've left our work. We've stayed [home]. We stopped what we were doing, and…you are not going to include us."

Similarly, data collectors said community members from rural areas asked why they were targeted, when people in urban areas were not.

…Others were saying why is it that you always come in the rural areas? When we phone our relatives in town, they say they don't experience such things.

There was also a question about why older individuals were being included, when they had not received the vaccine, as mentioned by this data collector:

Then the other question was, why are you selecting households, not collecting blood from everyone who was given an injection. Or that vaccine you are saying. And why are you collecting blood from everyone, including the oldest, when they didn't receive those vaccines.

These concerns about equity of selection did not arise in the caregiver survey.

Test results

The issue of test results was viewed as both a barrier and a facilitator, depending on the context. In the caregiver survey, fear of test results because they fear being told they have a disease was a substantial barrier to serosurvey participation (41%), yet all said receiving their test results was important to them (Table 7). The data collectors also noted the community valued receiving their test results. Although HIV testing was not specifically asked in either the

caregiver survey or as part of the qualitative data collection, it arose as an important theme. There were reports of participants wanting to be tested for HIV as well as refusals to participate in serosurveys for fear that they would be tested for HIV. Finally, the data collectors also mentioned privacy as a concern from participants who feared their test results would become known.

A sub-theme within test results focused on receiving test results. Receiving the results of any testing done on specimens was noted as important by both the data collectors and caregivers. All caregivers agreed that receiving test results was important to them, and a few said that not receiving their test results was a barrier to serosurvey participation. Additionally, 79% stated they were willing to accept summary-level results at the district level rather than individual test results.

Just as test results were reported as important to caregivers, the most commonly referenced requests from the qualitative data was the community wanting to know the results of the testing being done on their blood. Data collectors reported complaints from the community that data collectors in the past collected blood from them but did not provide test results. As noted by one supervisor from the serosurvey:

> *After sensitizing them, they gathered to refuse…say[ing] 'there are a lot of people coming to take our blood. They don't come back to tell us our results in person.' Everyone wants to know their results. Sometimes you say after pricking [you will get results], they say 'no, give us our blood back.' They say 'you are doing for everyone in this community. We want our results. You just come, you don't bring us anything.'*

A data collector also reported anxiety from the community over receiving their test results:

I think the community is also anxious to know their results. Because most of the time, these studies are being done, but the response is not taken back to the community.

One data collector also specifically noted that not receiving test results negatively affected the community's willingness to participate in the study:

The biggest question that we faced was…most of the time, we go to them to collect blood, but we don't tell them the outcome. When we collect blood from them, when we go, we [leave forever], without even informing them what we have found. So now they were like, 'you people, us we are very much willing to participate in this program. But the problem that we have is every time you come, you collect our blood, you go. But when it comes to giving us the results, you don't even tell us what you find.' That was their main concern.

A second sub-theme that emerged within test results focused on HIV testing. This sub-theme arose in both the caregiver survey and data collector qualitative data. Almost half of participants feared learning their test results, and some thought there could be stigma related to having their blood collected. While not explicitly stated by all, there were undertones of HIV testing in the open-ended responses. One caregiver mentioned long-term treatment despite lack of symptoms:

People scared of that they will be found with a disease and be put on treatment when they feel okay.

While another caregiver stated:

They fear to know their (HIV) status.

Finally, another caregiver specifically stated fear of stigma about HIV if they participate:

They do not want to be seen that they had their blood collected. Because then others will suspect that you have a disease such as HIV.

Similarly, data collectors also mentioned that people thought blood was being collected for HIV testing. In some cases, it was reported that community members wanted to get tested and had a desire to be included in the study to know their HIV status. As noted by one data collector:

People were running to the car and stopping the car, "we want to be tested for HIV." And we were saying, "no we are sorry. We don't have kits as for now, but you can go at the nearest clinic." And they said, "oh, we thought you were even testing for HIV." So we were just advising them to go at the clinic to have their HIV test. Otherwise they were just like, "no, we want HIV test." [We said] "No, it's not what we are after. We were after measles."

Conversely, data collectors also reported there were concerns from those asked to participate that their blood would be tested for HIV, despite being told this was not the case. Another data collector mentioned:

They would also think maybe if you don't [give] much information [about the study] that we are not testing HIV, they would think…you want to find out about our status. So it was vital again to introduce that, to say that, okay we are not testing this (HIV).

The final sub-theme within test results focused on privacy. There were community concerns noted by data collectors, related to test results being made public. This concern tended to arise during the informed consent process.

The question… "why are we signing the consent forms? Why are you taking our names?"

There was also a concern that their test result would become known. Participants pointed out that if the survey was anonymous, then names should not be required for participation.

> *So [they ask] questions like, "how am I going to know that you are not going to testing my blood?" Even the consenting part… [I would say] I've just written your first name. If you are Richard, imagine how many Richards. I've written here [participant identification number] just of those, it's just numbers. There was even this woman said, "but you asked for my name, then you're saying you won't publicize my rubella results. So why is my name for?"*

Specimen Preference

Specimen preference was specifically asked in the caregiver survey and not directly mentioned in the qualitative interviews. The survey demonstrated that participants tended to prefer finger pricks over venous blood draw (59%) and finger pricks over oral swabs (71%); however, there did not seem to be a strong preference between venous blood draw and oral swabs (Figure 6). General reasons for specimen preference included simplicity of the procedure, pain caused by the procedure, a desire to know their test results, and choosing a specimen based on the doctor's recommendation (Table 8). One-third of participants believed it was easier to have blood taken than saliva, while a few thought saliva was easier to collect. This could in part be due to participants saying they had never heard of testing from saliva. As noted by a caregiver, they knew diseases could be detected in blood:

> *The doctor can detect a lot of diseases in blood, but I don't know if he can detect anything in saliva.*

Similarly, another participant noted that they had experience with blood draws and finger pricks:

I have never had a mouth swab before so I wouldn't know how it works. I am familiar with a blood draw or pricks.

Overall, caregivers mentioned more familiarity with blood draws and finger pricks than with oral swabs.

Discussion

This study provides two perspectives on the acceptability of serosurveys in rural southern Zambia: the data collectors and potential serosurvey participants in the form of caregivers. We characterized the motivators and barriers behind why participants are willing to participate in serosurveys. As demonstrated by the socio-ecological model, individual level factors were not the driving motivators, but rather interpersonal relationships, and perhaps most importantly, structural issues related to serosurvey implementation. At the interpersonal level, familial input was important to decision making, and community healthcare workers were an important driver to participation because they provided information about the serosurvey and were trusted by the community. At the structural level, understanding use of specimen collection and provision of test results were key motivators for participation. Capitalizing on these motivators can help frame messaging for communities to improve participation in serosurveys.

The socio-ecological model framework and providing both the data collectors and participant perspectives in this study enabled us to better understand the drivers and barriers to participation (Protiere et al., 2017). We were able to note certain differences in themes that arose at the interpersonal and structural levels. Caregivers said that they were the most significant decision makers; whereas the data collectors thought community norms were a major influencer in the acceptability of the serosurvey, including religious and community leaders. At the

structural level, questions about equity of participation arose from data collectors, regarding who was being chosen to participate in the serosurvey, with some groups feeling targeted, while others felt excluded. There were also privacy concerns about test results becoming known that arose from the data collectors. These likely did not arise in the caregiver survey, as people were not actually being asked to participate in a serosurvey. Privacy concerns have been noted for other studies that require dried blood spot collection (Hendrix, Meslin, Carroll, & Downs, 2013). Similarities between data collectors and participants included religion being an important individual-level barrier, community health workers as a major interpersonal-level facilitator, and questions about specimen collection and not receiving test results as a barrier at the structural level. Whether HIV testing was being performed was also mentioned as both a facilitator and a barrier from both perspectives.

Addressing the barriers and facilitators to participation can reduce bias in serosurvey results. For example, it was noted that religion is a major barrier because Zionists and Seventh Day Adventists cannot provide blood specimens. If they are also groups that do not allow vaccination, the serosurvey will overestimate population immunity because they are not represented in the sample. Others who may not participate are those who are not home when sensitization occurs by the community health worker or when the actual survey is implemented. These could be households who work outside of the house or migrant families. In order to overcome this structural-level barrier, revisits or off-hour visits would need to be made to ensure they are included in the serosurvey (Salihu et al., 2015).

Selection bias may also occur in terms of motivation for participation in serosurvey, such as a desire to know test results. It was uniformly agreed that it was important for participating communities to receive test results, either individual results or community-level results. While

there were some contradictions in that participants feared receiving test results if they did not feel sick, this did not seem to be for measles and rubella testing. Provision of test results has been noted as a factor for participation in blood draws in clinical trials (Chatio et al., 2016). In HIV/AIDS and malaria research, where point of care tests are available, test results can be provided immediately; however, for measles and rubella this is not yet an option, so other means of providing feedback would be required. For this reason, we asked if providing community-level feedback would be acceptable, and 79% stated this would be amenable to them. Options to providing community feedback could include community-wide meetings, or meetings with community leaders or community health workers to provide aggregate results.

As noted by both the data collectors and caregivers, there were participants who were both encouraged to participate by HIV testing, as much as there were participants who were discouraged from participating because they did not want to be tested for HIV, despite HIV testing not being part of the testing procedure for the serosurvey. Similar concerns of HIV testing of biological specimens have been noted in clinical trials in Africa (Chatio et al., 2016). Given the logistic and ethical issues with providing HIV testing, understanding the preferences of the community on HIV testing would be important to balance acceptability and feasibility of serosurveys.

Although the focus of this paper is on the acceptability of serosurvey participation, there were some issues that arose that are generally applicable to all household surveys. In Table 7, most of the barriers named by caregivers were specific to serosurveys; whereas the influences listed as facilitators were general survey participation recommendations to improve acceptability. Similarly, all the interpersonal influences on serosurvey acceptability were general survey participation issues. Using a community health worker who the community knows and trusts has

been associated with increased likelihood of participation in research (Okello et al., 2012; Salihu et al., 2015) . Similarly, fear and distrust of research are barriers to research participation (Moreno-John et al., 2004). At the structural level, selection criteria for participation is also applicable to surveys in general.

By contrast, the issues that were specific to serosurveys were religion preventing specimen collection at the individual level, and test results and questions about specimen collection at the structural level. Not knowing what would be done with blood specimens is a common barrier to research participation (Nakalembe, Mutyaba, & Mirembe, 2016). As noted by caregivers, the most frequently provided solution to overcome questions about specimen collection was to provide information to the community, which is a facilitator for all surveys. Religion as an individual barrier to participation can be overcome through community education, including engagement of religious leaders (Machekanyanga et al., 2017). The second most noted facilitator was to gather everyone in one location for specimen collection, which is specific to serosurveys. Because blood collection can be viewed as a medical procedure, conducting it at the household may not be as acceptable as doing so in a more central, sterile location (Ostermann et al., 2015). Understanding how much dropout occurs when blood collection is added to the survey, as compared to what dropout would have been without blood collection is an important consideration for serosurveys.

In terms of specimen preference type, finger prick blood collection was notably preferred by caregivers. This finding is consistent with another study in Zambia that also reported preference for finger prick over venous blood collection by both participants and providers (Sutcliffe et al., 2017). By contrast, a study in Tanzania found that community members preferred venipuncture over finger prick blood collection (Ostermann et al., 2015). Our study

results indicated that blood was likely preferred because participants knew it as an effective means of detecting disease; whereas saliva was unfamiliar to most participants. The study population opinions are likely influenced by the fact that there are programs and research studies monitoring HIV/AIDS and malaria in the area (Columbia University, 2016; Larsen et al., 2015). Therefore, participants may be accustomed to receiving finger pricks for rapid testing, as noted by one third of caregivers having participated previously in a survey with specimen collection. The question of self-collection of finger prick blood was also asked but needs further study, as this was theoretical and not asked of every participant. This has been explored for HIV self-testing, where users generally found self-finger pricking to be acceptable (Knight et al., 2017).

Although we provide the community perspective by having surveyed caregivers directly, they are not caregivers who had necessarily participated in a serosurvey. They were asked theoretical questions about whether they would participate; though 30% had participated and there was no difference between the responses of those who had versus those who had not previously participated in serosurveys. Furthermore, there could be selection bias, as caregivers were recruited at health facilities, which may make them more likely to accept specimen collection because they engage with the healthcare system. Almost all the caregivers who had participated in previous surveys with specimen collection were for HIV/AIDS or malaria. There was also a prominent undertone of HIV/AIDS concerns underlying the responses in this population, such as fear of their 'status' or being put on treatment when they do not feel sick. Whether this is due to direct association with blood specimen collection, previous serosurvey for HIV, or social mobilization campaigns is not known. Finally, these results reflect a setting with high HIV and malaria transmission, which is accustomed to blood collection for diagnostic purposes, so our findings may not be generalizable to other settings. Despite these limitations,

our study is among the first to elucidate factors, both facilitators and barriers, to serosurvey acceptability.

Conclusion

Overall, serosurvey participation was deemed acceptable to most study participants. Refusals can be minimized by understanding the motivators and facilitators in the community in order to prevent high refusal rates. The socioecological model reveals barriers and facilitators for participation to guide strategies to improve participation. Specifically, planned serosurveys should make provisions to provide information about blood collection ahead of the serosurvey and communicate through a trusted vehicle such as the community health worker. If assessment is done to evaluate context-specific influencers of acceptability, it could help to guide strategies, improve participation, and reduce bias in a serosurvey.

Tables and Figures

Table 5. Characteristics of data collectors who participated in in-depth interviews and focus group discussions

	n=24	%
Role		
Supervisors	11	45.8
Data collectors	13	54.2
Male	8	33.3
Districts covered per team		
Kazungula, Livingstone	6	25.0
Choma, Kalomo, Namwala	5	20.8
Choma, Pemba	4	16.7
Gwembe, Monze	6	25.0
Province level	3	12.5

Table 6. Sociodemographic factors and serosurvey response characteristics of caregivers who participated in the survey

District	n=34	%
Namwala	12	35.3
Kazangula	11	32.4
Choma	11	32.4
Religion		
Catholic	7	20.6
Pentecostal	3	8.82
Protestant	4	11.8
Seventh Day Adventist	13	38.2
Other	7	20.6
Time to vaccination clinic		
<30 minutes	8	23.5

30-59 minutes	10	29.4
60+ minutes	16	47.1
Who influences decision making*		
Self	23	67.65
Family	14	41.18
Healthcare worker	5	14.71
Community healthworker	2	5.88
Chief or leader	0	0
Participated in surveys	n	%
No	24	70.6
Yes		
If yes, type of survey:	10	29.4
HIV	4	40.0
Malaria	5	50.0
Sanitation	1	10.0
Doesn't remember	2	20.0
Bodily fluid collected	8	80.0
Results received	5	62.5
Number of surveys in which participated		
0	1	10.0
1	6	60.0
2-4	3	30.0
Willingness to participate in future serosurveys		
Willing to allow finger prick	25	73.5
Willing to allow child's finger prick	21	61.8

Notes: (*) Participants could name multiple sources of influence

*Table 7. Caregiver barriers and facilitators to serosurvey participation (n=34)**

Barriers	n	%
Unsure what is being done with blood	18	52.9
Religious beliefs	15	44.1
Scared to know results	14	41.2
Not getting test results	4	11.8
Fear of needles	4	11.8
Stigma	3	8.8
Satanism	2	5.9
Safety concerns	2	5.9
Research fatigue	1	2.9
Facilitators		
Information provided	29	85.3
Have people come to central location for serosurvey	16	47.1
Someone community trusts (like CHW)	10	29.4
Desire to know about health issues	10	29.4
Community thinks they will get a benefit	4	11.8
Go house to house for serosurvey	1	2.9
Desire to better society	1	2.9

Notes: (*) Participants could name multiple barriers and facilitators

Table 8. Reasons for preferring a specimen type (n=34)

Reason for preference*	n	%
Simplicity of procedure	8	23.5
Pain from procedure	8	23.5
Desire to know test results	7	20.6
Doctor recommendation	5	14.7
Amount of blood	4	11.8
Blood is better than saliva	9	26.5
Saliva is better than blood	3	8.8
Never heard of saliva testing	5	14.7

Notes: (*) Participants could provide multiple reasons for their specimen preference

Figure 5. Emerging themes related to acceptability of a serosurvey within a socioecological model

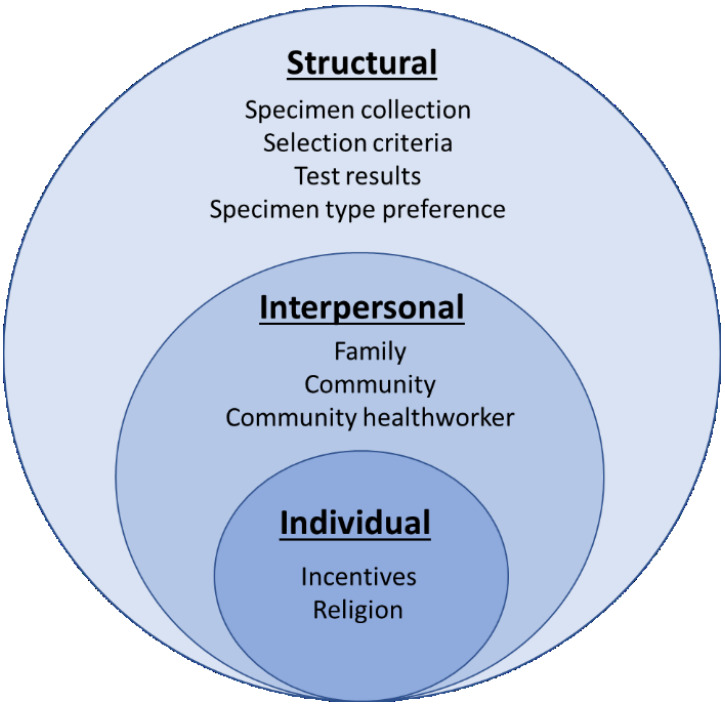
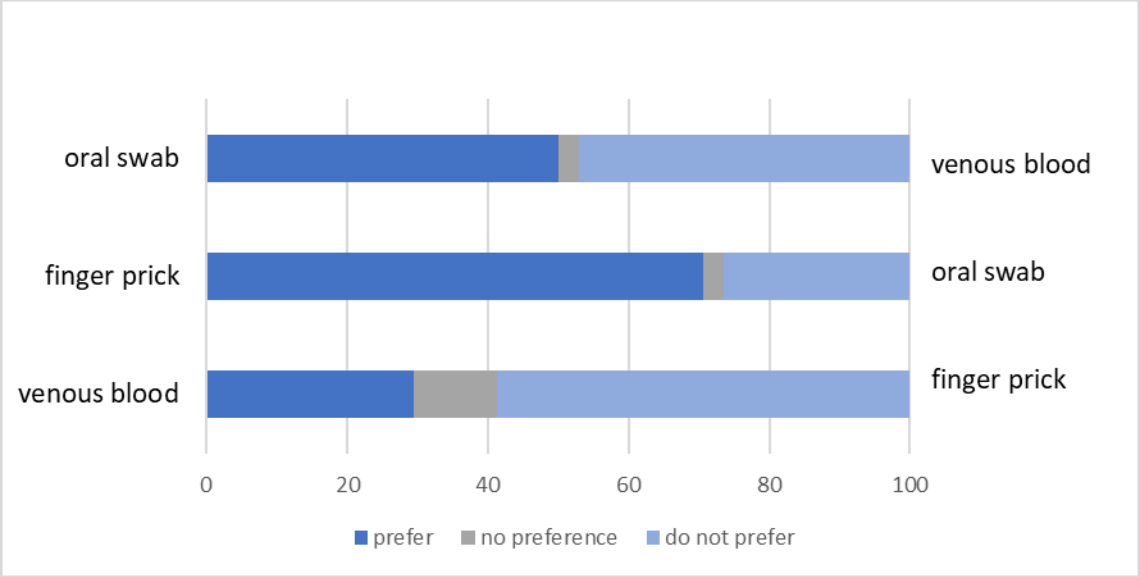


Figure 6. Pairwise specimen preference among caregivers comparing oral swab, venous blood and finger prick collection methods



Notes: (n=34) Participants were asked pair-wise specimen preference types, and stated either: preferred, no preference, or did not prefer one specimen over another. Only one response allowed per pairwise comparison.

Paper 2- How much does it cost to measure immunity? A costing analysis of a measles and rubella serosurvey in Zambia

Abstract

Background: Serosurveys provide a more direct measurement of population immunity to infectious diseases such as measles and rubella than vaccination coverage; however, there is concern that serosurveys are costly. To better understand the costs associated with conducting a serosurvey, we adapted a framework that includes calculating personnel, data collection and laboratory costs.

Methods: We costed a nested serosurvey that was conducted in Southern Province that involved collecting dried blood spots from households in a post-campaign vaccine coverage survey. The financial costs were estimated using an ingredients-based costing approach from the government-funded healthcare perspective over a two-month horizon. Program cost data were obtained from the serosurvey budget, program records and interviewing administrative personnel. Inputs included personnel, transportation, field consumable items, social mobilization, laboratory supplies, and capital items, and were classified by serosurvey function (survey preparation, data collection, biospecimen collection, laboratory testing, coordination). Inputs were also stratified by whether they were general to surveys or attributable specifically to serosurveys. Finally, we calculated the marginal cost per cluster and participant to vary serosurvey sample sizes and estimate the effect on cost.

Results: We estimated that the total serosurvey cost in Southern Province, Zambia was US $68,558 to collect dried blood spots from 658 participants. Personnel costs were the largest contributing input to overall serosurvey costs (51%), transportation was second (23%), and field

consumables was third (9%). Data collection in the field accounted for almost one third of the total serosurvey cost (32%), followed by survey preparation (25%) and biospecimen collection (20%). By combining the serosurvey with the vaccination coverage survey, there was a savings of $43,957. In our nested serosurvey, the cost ratio (marginal cluster cost/marginal participant cost=$1,620/$30) was 54, reflective of a much higher cost to add a cluster as compared to adding an additional participant within an existing cluster.

Discussion: We estimated that the added time for biospecimen collection in the field and laboratory-related supplies were only 36% of the overall serosurvey budget. Similarly, our survey identified personnel and transportation as the highest cost inputs; however, we were able to save $26,539 in personnel and $13,410 in transportation by nesting within the post-campaign vaccine coverage survey. Using an existing biorepository rather than collecting new biospecimens could result in cost-savings in the areas of data collection and biospecimen collection, which together account for more than half the cost of the serosurvey. Understanding what drives the cost of serosurveys sheds light on how costs could be minimized.

Introduction

Monitoring population immunity to measles and rubella viruses can help identify populations at risk of outbreaks and determine whether targeted vaccination efforts are needed. While vaccination coverage may be used to approximate population immunity levels, vaccinated individuals can remain susceptible and unvaccinated individuals can be immune following infection. Serosurveys provide a more direct measurement of population immunity to infectious diseases such as measles and rubella (Cutts & Hanson, 2016). With the establishment of many regional measles and rubella goals by 2020, the use of serosurveys to identify population immunity gaps has been increasing globally (World Health Organization, 2015). However, there is concern that serosurveys are costly and time consuming (Metcalf et al., 2016). WHO measles and rubella serosurvey guidelines are currently being developed and suggest it could cost from $100,000 to over $1 million and require a one-year timeline to conduct a serosurvey (Patel, 2016). Understanding what drives the cost of serosurveys can shed light on how costs could be minimized. While many serosurveys have been conducted in developed countries, reducing cost could make them more accessible to low- and middle-income countries (Thompson & Odahowski, 2016).

Some of the costs of serosurveys would be required for any survey, regardless of whether they include biospecimen collection or not, such as vaccination coverage surveys or Demographic & Health Surveys (Keogh, 2005). These include data collection costs such as travel and fieldwork including participant enrollment and questionnaire administration. (Luman et al., 2007). Adding specimen collection to a planned survey could result in cost savings; however there are concerns about logistical feasibility, cost implications, and survey implementation (MacNeil et al., 2014).

Another major driver of cost is the sample size of a survey, which is influenced by the prevalence of what is being estimated and the precision of the estimate (Turner et al., 1996). Sampling strategies contribute to the cost of surveys differently, and understanding the implications of sampling on serosurveys could help determine their feasibility (Parker & Cubitt, 1999). For example, if biospecimens could be collected from a subset of participants within a larger survey, this could help minimize costs.

On the other hand, there are some costs that are unique to serosurveys because they require biospecimens. Serosurveys can prospectively collect biospecimens in the field or use specimens that have already been collected, such as a biorepository or residual samples. While prospective specimen collection allows for better control of data collection and sampling methodologies, collecting, processing and transporting blood specimens can be expensive and logistically challenging (Bryant Borders et al., 2007; Metcalf et al., 2016).

One of the reasons that biospecimen collection can be challenging is that it can require trained and skilled workers or healthcare professionals. This can vary depending on the type of biospecimen collected. For example, finger prick blood collection can be done by trained community health workers; whereas venous blood collection would require a phlebotomist or healthcare professionals (Harvey et al., 2008; World Health Organization, 2010). Biospecimens will always require specimen collection supplies, storage and transport, laboratory testing, and additional human resources for laboratory processing and testing (MacNeil et al., 2014).

To better understand the costs associated with conducting a serosurvey, we adapted a framework developed to cost integrated vaccine-preventable disease surveillance that includes calculating personnel costs and laboratory costs (Toscano et al., 2013). We populated the

framework with data from a serosurvey conducted in Southern Province, Zambia to examine how different components of the serosurvey affect the total cost.

Methods

Study location and design

This study was conducted in Zambia, where measles and rubella remain endemic, and cases continue to be reported annually (Zambian Ministry of Health, 2019a). Following a measles-rubella vaccination campaign in 2016 for children younger than 15 years of age, a national post-campaign vaccination coverage survey was conducted. In conjunction with this survey, a nested serosurvey was conducted in Southern Province that involved collecting biospecimens from all members of households enrolled in the post-campaign vaccine coverage survey. Dried blood spots were collected by fingerprick for enrolled individuals (Hayford et al., 2019). The WHO vaccination survey manual was used for the household-based survey design (World Health Organization, 2018b). Clusters were defined as administrative boundaries known as Supervisory Enumeration Area based on the most recent census conducted in 2010. Each cluster was selected to enroll 12 children who were eligible at the time of the vaccination campaign. Only 16 of the 26 clusters selected for the coverage survey in Southern Province participated in the serosurvey for logistical reasons.

Cost data

The financial costs were estimated using ingredients-based costing within activities approach, except for missing data, for which gross expenditure costing was used (Levin & McEwan, 2001; McEwan, 2012). The cost analysis was performed from the government-funded

healthcare perspective, where a non-government organization was hired as the implementing partner. We did not consider participant costs. Cost data were captured in local currency and converted into US dollars using the annual exchange rate in 2016 (USD $1=10.3 Kwacha) (World Bank, 2018). We considered a two-month time horizon for implementation of the serosurvey, from planning to laboratory processing. Weighting for incremental costs and discounting were not performed. Serosurvey cost data were obtained through document review of the budget and program records (such as purchase orders and contracts) and interviewing administrative personnel. When there was a difference between the budget and reported expenditure, reported expenditure was used to more accurately reflect the serosurvey as it was implemented rather than designed (McEwan, 2012).

Serosurvey cost estimation

A costing framework for integrated disease surveillance was adapted to capture the categories of implementation inputs across serosurvey functions (Figure 7). Serosurvey functions captured the phase of implementation from planning to data and biospecimen collection in the field and laboratory testing. Cross-cutting items such as communication, supervision, and data management were placed in a separate category of coordination, as they spanned serosurvey functions. The framework was compared with Demographic Health Services and vaccination coverage survey budgets to ensure all costs were captured (Keogh, 2005). For each category, we identified the proportion of costs attributed to a traditional vaccination coverage survey and those that were specific to the serosurvey. Costs were also stratified by whether they varied at the study, cluster or participant level to allow calculation of marginal costs (Yansaneh, 2005).

Personnel

We recorded the number of workers required for each activity. Long-term personnel who temporarily supported the serosurvey were allocated the time they spent doing serosurvey activities, including benefits. Personnel who were contracted specifically for the serosurvey had all their time included. We also included fees of consultants who supported specific services such as training. We apportioned total personnel costs to serosurvey activities based on the ratio of time spent doing serosurvey-specific activities (e.g., blood collection) compared to general survey activities (e.g., mapping). For the base case, one team was assumed to complete the serosurvey in one cluster within three days. The team consisted of a supervisor, phlebotomist, and three data collectors.

Transportation

No vehicles were purchased for the serosurvey; however, existing vehicles charged vehicle-use and driver time. Some vehicles were also rented at a fixed per day cost for vehicle use and driver time. Most transportation cost was allocated to general survey activities. Only biospecimen collection trips were included in serosurvey-specific costs. Each team was assigned one vehicle to implement field activities for the serosurvey.

Field consumables

Field consumables included the cost of any items required during data and biospecimen collection and was stratified by items required for general survey implementation, such as pens, and serosurvey-specific items, such as biospecimen collection kits (e.g. lancets, cotton, gloves). General survey items typically were calculated per cluster as they were team-level costs. Serosurvey specific items were typically calculated per participant.

Social mobilization

Because social mobilization for the post-campaign vaccine coverage survey was done as part of the vaccination campaign, all additional social mobilization efforts were calculated as serosurvey-specific costs. This included stipends for community health workers to accompany the teams in the field during data collection, radio ads, and meetings and phone calls with health facility staff to notify them that a serosurvey was being conducted in their area.

Laboratory supplies

Laboratory costs were calculated based on the cost of supplies and consumable materials (e.g. test kits, gloves, tubes). The cost of bench space was based on the time required for laboratory testing. We assumed a laboratory providing services for a serosurveys has the equipment to conduct enzyme immunoassays using commercial kits; therefore we did not account for equipment or cost of maintenance. It was assumed that 15% of biospecimens would be retested in the base case to account for quality assurance and quality control, as well as retesting biospecimens with equivocal results and biospecimens with results above the upper limit of detection for a commercial measles and rubella IgG enzyme immunoassay kit.

Capital items and overhead

Because we costed a single serosurvey, the only capital items purchased specifically for the serosurvey were tablets for data entry. All other equipment was borrowed, and use-time was included in the cost. No overhead costs were included.

Data analysis

We used Microsoft Excel to compile and analyze the data. Using the number of clusters and participants included in the serosurvey, we calculated the average per cluster and per

participant costs, as well as the marginal cost per cluster and participant. Estimated costs were then stratified by serosurvey activity. Inputs were also stratified by whether they were general to surveys or attributable specifically to serosurveys. One-way sensitivity analyses were conducted for personnel and time spent on various activities.

Marginal costs for an additional cluster were calculated based on social mobilization, personnel, team materials and transportation required to include a cluster in the survey. This included one day for enumeration, mapping, and social mobilization preparatory activities required at the cluster. Marginal costs for an additional participant in an existing cluster included consumables for biospecimen collection and laboratory testing as well as additional time for personnel and transport.

These marginal costs were used to estimate the cost of conducting a similar survey under different assumptions. Sample sizes were estimated using the Expanded Program on Immunization (EPI) cluster survey methodology for varying population immunity levels, precision, and design effects (World Health Organization, 2018b). Precision around the point estimates were modified for the sample sizes by changing both number of clusters and number of participants per cluster. These sample sizes were then costed using the marginal costs per participant and clusters to estimate the cost of conducting these serosurveys at varying precision and assumed population immunity levels. We assumed similar underlying seroprevalence across clusters for these analyses.

Results

Biospecimens were collected from 658 individuals in 16 clusters by four teams comprised of 5 members each (supervisor, interviewers, and blood collector). Each team took

approximately 3 days to complete each cluster. One and half weeks were calculated for training and piloting, and 3 weeks were required for laboratory testing. With these assumptions, we estimated that it cost US $68,558 to collect biospecimens in a household vaccination coverage survey in Southern Province, Zambia. This resulted in a cost of $4,285 per average cluster and $104 per average participant sampled.

Personnel costs were the largest contributing input to overall serosurvey costs (51%), transportation was second (23%), and field consumables was third (9%) (Figure 8). A breakdown of the cost by serosurvey activity is presented in Table 9. Data collection in the field accounted for almost one third of the total serosurvey cost (32%), followed by survey preparation (25%) and biospecimen collection (20%). The overall added cost of collecting and testing a biospecimen as part of the survey was $24,601 (36%). In terms of costs attributable exclusively to serosurveys, personnel costs were also the largest input (34%) due to added time in the field and laboratory personnel, followed by laboratory supplies (26%) and social mobilization (18%). By combining the serosurvey with the vaccination coverage survey, there was a savings of $43,957, the cost of completing just the vaccination coverage survey.

We estimated that the marginal cost of including an additional cluster was $1,620, and the marginal cost of adding a participant within an existing cluster was $30. For these, 46% of the cost to add a cluster and 64% of the cost to add a participant were attributable to serosurvey-specific costs. In the serosurvey, all members of the household including both adults and children were enrolled, whereas only children were included in the vaccination coverage survey. Incorporating more than one child per household, comprising an additional 235 children, added $7,030 to the serosurvey cost; including 219 adults in the serosurvey cost an additional $6,552.

Few participants were seronegative for measles or rubella, as the serosurvey was conducted after an immunization campaign. Given that 33 participants were measles seronegative, the average cost per seronegative identified was $2,077. There were 18 participants seronegative for rubella, resulting in an average cost of $3,809 per seronegative identified. Accounting for both measles and rubella seronegatives, it cost $1,344 per susceptible identified.

Sensitivity analyses show that varying time for field work and number of team members had the largest impact on serosurvey cost, as this also affected field work time (Figure 9). These factors impacted the data collection phase, which accounted for most of the study's costs. However, none of the sensitivity analyses changed the cost by more than 8%.

Costs associated with varying sampling design

Additional sensitivity analyses were conducted to evaluate how varying the sampling design of the serosurvey affected cost. Our base case of the nested serosurvey used to estimate costs had an average of 43 participants enrolled per cluster (SD=17). The intraclass correlation was 0.026 for measles with a mean seroprevalence of 96.5% (SD=5.1) and 0.005 for rubella, with a mean seroprevalence of 95% (SD=6.0). The sample size conditions for the nested serosurvey assumed 85% estimated population immunity with +/-7% precision and a design effect of 1.4, which would have required 16 clusters with 12 participants per cluster. Varying sampling parameters had differential effects on the cost of a serosurvey.

Estimating seroprevalence in settings where it is assumed to be high has lower associated costs than settings with lower seroprevalence due to the decreased sample size required to obtain a point estimate with a specified precision, and thus the range of costs is smaller (Figure 10). The design effect (DEF) can also have substantial implications for cost, with a higher DEF

having a higher cost. For example, 85% population immunity with 5% precision and 10 clusters can cost $76,684 when the DEF=1.4 or $99,709 when DEF=2.0 due to the increased number of participants needed with higher design effect (Figure 11).

While the effective sample size for improving precision around a point estimate will always increase, how the cost of the larger sample size increases depends on the marginal cluster cost and marginal participant cost. In our nested serosurvey, the cost ratio (marginal cluster cost/marginal participant cost=$1,620/$30) was 54, reflective of a much higher cost to add a cluster of average size compared to adding an additional participant within an existing cluster (Kish, 1965). Our average cluster size was 42 participants. Assuming 85% seroprevalence and that all clusters have similar seroprevalence, improving from +/-10% precision to +/-5% by keeping the number of participants the same and adding clusters resulted in a cost increase of $33,636 (Table 10). Whereas improving the same precision by maintaining the number of clusters and adding participants within a cluster, resulted in a cost increase of only $6,225. (Table 11). Figure 12 demonstrates that for the 85% estimated population immunity and 5% precision, the total number of participants required would be 275, which can be achieved with varying combinations of number of geographic clusters and participants at varying cost, assuming underlying immunity is similar across clusters. Using this figure, if you had $60,000 to conduct a serosurvey, you would be limited to doing so in 17 or fewer clusters.

Discussion

Economic evaluations of public health surveillance systems have used various methodologies, making it difficult to compare across settings (Herida, Dervaux, & Desenclos, 2016). To compare serosurvey costs across settings, we developed a framework for serological

surveillance and used it to estimate the costs of a nested serosurvey in Southern Province, Zambia. Standardized categories permit cross-country comparisons and could be used as a model for other countries considering serological surveillance.

Few costing studies of serological surveillance have been conducted, and to our knowledge, none have been done for measles and rubella serosurveys. By nesting this serosurvey within a planned post-campaign vaccine coverage survey, costs for planning, personnel, mapping, enumeration, and transportation were not borne by the serosurvey. WHO estimated that 60-70% of a serosurvey budget is laboratory-related supplies for blood collection, storage, transport, processing, and testing kits (World Health Organization, 2015). We estimated that the added time for biospecimen collection in the field and laboratory-related supplies were only 36% of the overall serosurvey budget. This could be due to the limited serosurvey transportation cost, as we did not have vehicles allocated specifically for biospecimen transport. Another potential explanation for these differences could be the biological specimen type. Since we used fingerpick blood collection, this may have reduced the costs of biospecimen collection, as it does not require personnel skilled in venous blood draw. Collecting biospecimens as dried blood spots eliminated the need for a reverse cold chain and time-sensitive transportation to the laboratory.

Although the MR vaccination campaign targeted children younger than 15 years of age, monitoring seroprevalence in adults is important because as measles and rubella virus circulation diminishes, fewer people are immunized through natural infection in childhood and will be at risk of acquiring disease as adults. This is of particular concern for women of childbearing age, as it could result in increased congenital rubella syndrome cases (Lessler & Metcalf, 2013). In this serosurvey, lower rubella seroprevalence (88%) was identified in women 16 to younger than 30 years of age, the age group in which protection from rubella is critical to prevent CRS. It cost

an additional $6,552 to include the 219 adults in households were children were already being enrolled. The serosurvey revealed immunity gaps among young adults not eligible for the campaign, gaps which would not have been identified through the vaccination coverage survey alone. This adult population can be monitored through serological surveillance without a substantial increase in funds required.

Understanding the factors that have the greatest effect on serosurvey cost can be used to minimize these costs. Other household surveys have noted that cost of survey implementation in sub-Saharan Africa is often higher than in other regions due to high personnel costs (Kilic, 2017). An EPI costing study in Zambia also identified personnel and travel as the highest implementation costs for routine immunization (Schutte et al., 2015). Similarly, our survey identified personnel and transportation as the highest cost inputs; however, we were able to save $26,539 in personnel and $13,410 in transportation by nesting within the post-campaign vaccine coverage survey.

Using an existing biorepository rather than collecting new biospecimens could result in cost-savings in the areas of data collection and biospecimen collection, which together account for more than half the cost of the serosurvey. Other ways to reduce costs include limiting training time by using experienced data collectors and improving data collection tools to minimize time in the field.

As new technology continues to develop such as point-of-care technology to detect antibodies, laboratory costs could be affected. These technologies might be more expensive than current laboratory methodologies, but they may not require specimen transportation and could be done by non-laboratory personnel which could decrease costs (Vojnov et al., 2019). The use of multiplex bead arrays to detect multiple antigens from the same biospecimen, could make

serosurveys more cost-effective by providing information on multiple diseases in less time without requiring additional biospecimen collection (Itell et al., 2018).

The cost for identifying someone who is seronegative was high in this population because of the high seroprevalence and thus few seronegative individuals. The cost per seronegative individual will vary across settings and would be lower where seroprevalence is lower. By contrast, it is cheapest to conduct a serosurvey, with the objective of obtaining a seroprevalence estimate, in settings where seroprevalence is higher because of the decreased sample size required. The objective of the serosurvey in this case is important to understand. For example, if a serosurvey is being conducted to verify elimination status, or confirm the effectiveness of a vaccination campaign, high seroprevalence would be expected. Similarly, if an area has high routine immunization coverage, high seroprevalence would be expected. If a country were prioritizing areas for vaccination campaigns, a serosurvey could be conducted in the area to confirm high seroprevalence, allowing the country to consider not vaccinating in that area, but rather focus on areas where seroprevalence is low. On the other hand, conducting a serosurvey in areas with low expected seroprevalence would more expensive because of an increased sample size needed, but would result in the identification of more people who are seronegative who could be targeted for vaccination campaigns.

As demonstrated by the sample size estimates, the cost of increasing precision varies greatly depending on design effect, estimated population immunity, and number of clusters. Achieving additional precision by adding participants within clusters had less of an impact on cost, as the cost of including an additional cluster far outweighs the cost of including an additional participant within that cluster. However, adding additional participants in a homogenous cluster does not provide additional information. Careful consideration of when

inclusion of another cluster is needed will depend on the heterogeneity between clusters, and whether the information gained from adding an additional cluster warrants the added cost (Pettersson, 2015).

Limitations

Since the data sources were requested a year after the study was completed, the uncertainty in cost estimates may vary based on quality of record keeping (McEwan, 2012). Because this was a one-time activity, new personnel were not hired, but rather contracted for their time. If this were to be an ongoing activity, hiring additional personnel or allocating a proportion of existing personnel time would be needed. Transportation costs were based on per day use rather than actual mileage covered. We were not able to estimate the marginal cost per household due to the format of the costing data and the variable number of people included per household; therefore, estimates were based on participant costs. These costing estimates were for the actual number of participants enrolled and may not account for additional time required if conducting a serosurvey in a setting with high refusal rates or non-response that would require additional time spent on enrollment.

Similarly, sample size estimates demonstrate the relationship between precision around an estimate and cost, but they do not consider the potential for bias. The cost of conducting the serosurvey decreases with fewer clusters but sampling too few clusters could increase the potential for bias. Our intraclass correlation for the base case used to cost the study was low, but in a setting with higher intraclass correlation, there would be a higher design effect. The implications for the number of clusters to include would be important to provide an accurate

seroprevalence cost estimate. Balancing the desire to lower cost and the precision of the seroprevalence estimate could be simulated using varying seroprevalence estimates per cluster.

Conclusion

Adding serological specimen collection to a planned vaccination coverage survey in Southern Province, Zambia provided a more direct measurement of population immunity, while increasing the cost by approximately one-third. Vaccination coverage can provide an estimate of how many children were vaccinated but does not consider non-response to vaccination or natural infection, therefore not providing true population immunity. Instead, serosurveys can directly measure population immunity and be done efficiently by nesting the serosurvey within a vaccination coverage survey to save costs. Strategies in cluster survey design methodology can help identify ways to minimize costs. Future serosurveys could consider ways to leverage existing surveys conducted for other purposes to minimize costs.

Tables and Figures

Table 9. Costs per phase of serosurvey implementation in Southern province, Zambia

Serosurvey phase	Cost (2016 USD)	Percentage of total cost
Survey preparation	$ 16,813	25%
Data collection	$ 22,062	32%
Biospecimen collection	$ 13,875	20%
Laboratory testing	$ 10,726	16%
Coordination (Communication, Data management)	$ 5,081	7%
TOTAL	$ 68,558	

Notes: Phases of the serosurvey correspond to Figure 7. Percentages sum to 100%. All costs in 2016 USD.

Table 10. Sample size costing with varying precision by changing the number of clusters

Seroprevalence estimate (%)	Margin of error (%)	Number of clusters	Participants per cluster	Total Cost
65	+/-10	11	12	$ 44,724
	+/-7	21	12	$ 64,510
	+/-5	41	12	$104,082
75	+/-10	9	12	$ 40,767
	+/-7	18	12	$ 58,574
	+/-5	34	12	$ 90,231
85	+/-10	6	12	$ 34,831
	+/-7	12	12	$ 46,703
	+/-5	23	12	$ 68,467
95	+/-5	9	12	$ 40,767

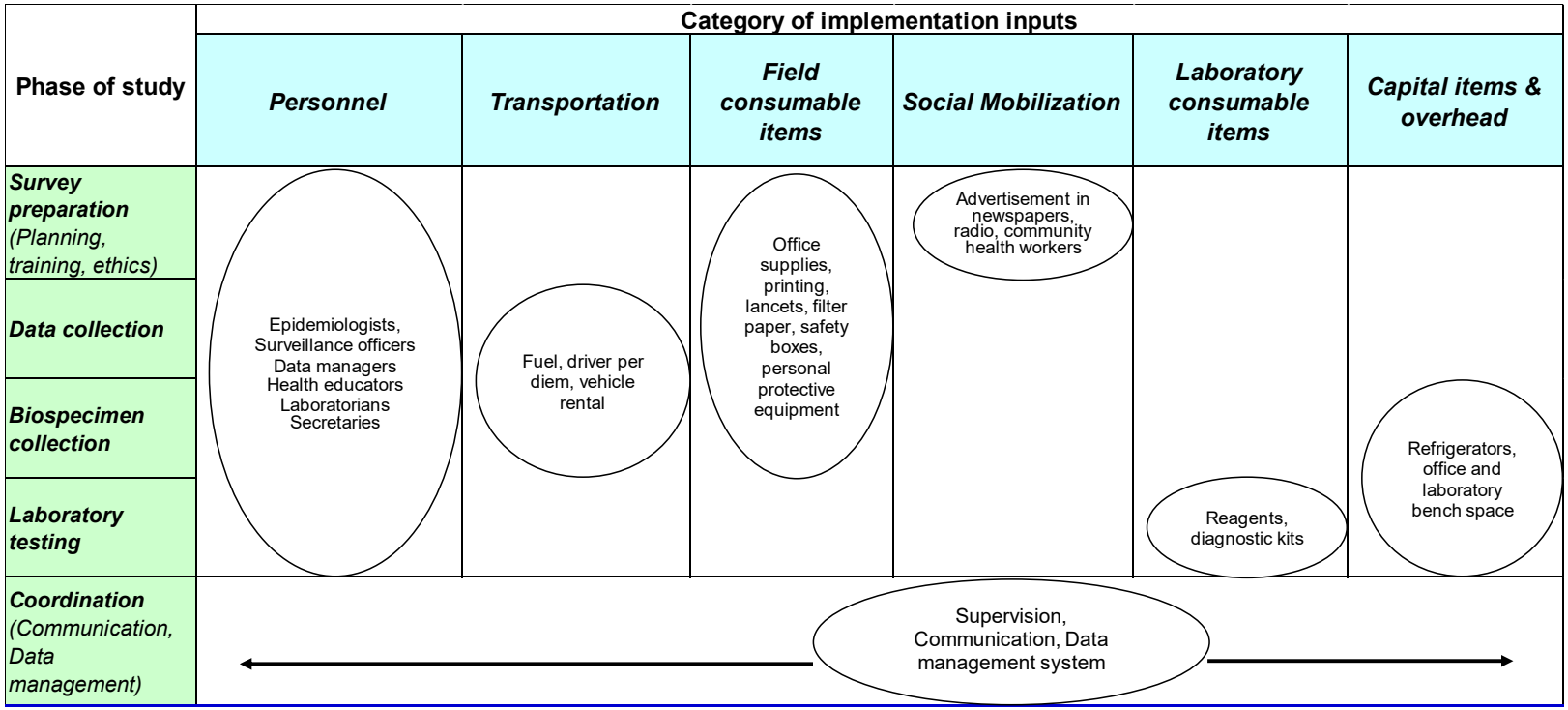
Notes: Sample sizes are calculated for corresponding assumed seroprevalence estimate, margin of error, number of clusters, and number of participants per cluster. Assumed DEF=1.4, alpha=0.05, and one participant per household. Total cost= base cost ($22,960) + number of clusters*marginal cluster cost ($1,620) + number of participants per cluster *number of clusters* marginal cluster cost ($30). All costs in 2016 USD.

Table 11. Sample size costing with varying precision by changing the number of participants per cluster

Seroprevalence estimate (%)	Margin of error (%)	Number of clusters	Participants per cluster	Total Cost
65	+/-10	16	8	$ 52,695
	+/-7	16	16	$ 56,526
	+/-5	16	31	$ 63,709
75	+/-10	16	7	$ 52,216
	+/-7	16	13	$ 55,089
	+/-5	16	26	$ 61,315
85	+/-10	16	5	$ 51,258
	+/-7	16	9	$ 53,174
	+/-5	16	18	$ 57,484
95	+/-5	16	7	$ 52,216

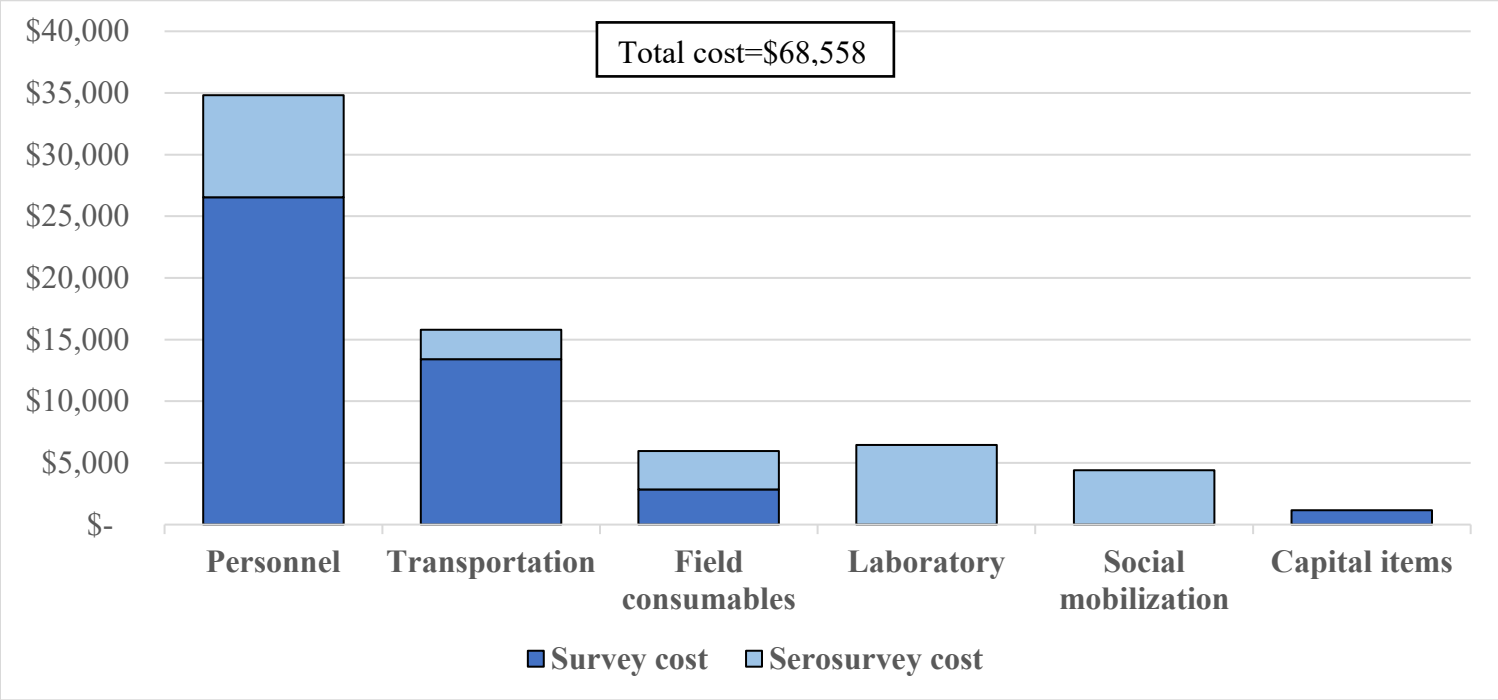
Notes: Sample sizes are calculated for corresponding assumed seroprevalence estimate, margin of error, number of clusters, and number of participants per cluster. Assumed DEF=1.4, alpha=0.05, and one participant per household. Total cost= base cost ($22,960) + number of clusters*marginal cluster cost ($1,620) + number of participants per cluster *number of clusters* marginal cluster cost ($30). All costs in 2016 USD.

Figure 7. Framework for estimating serosurvey costs



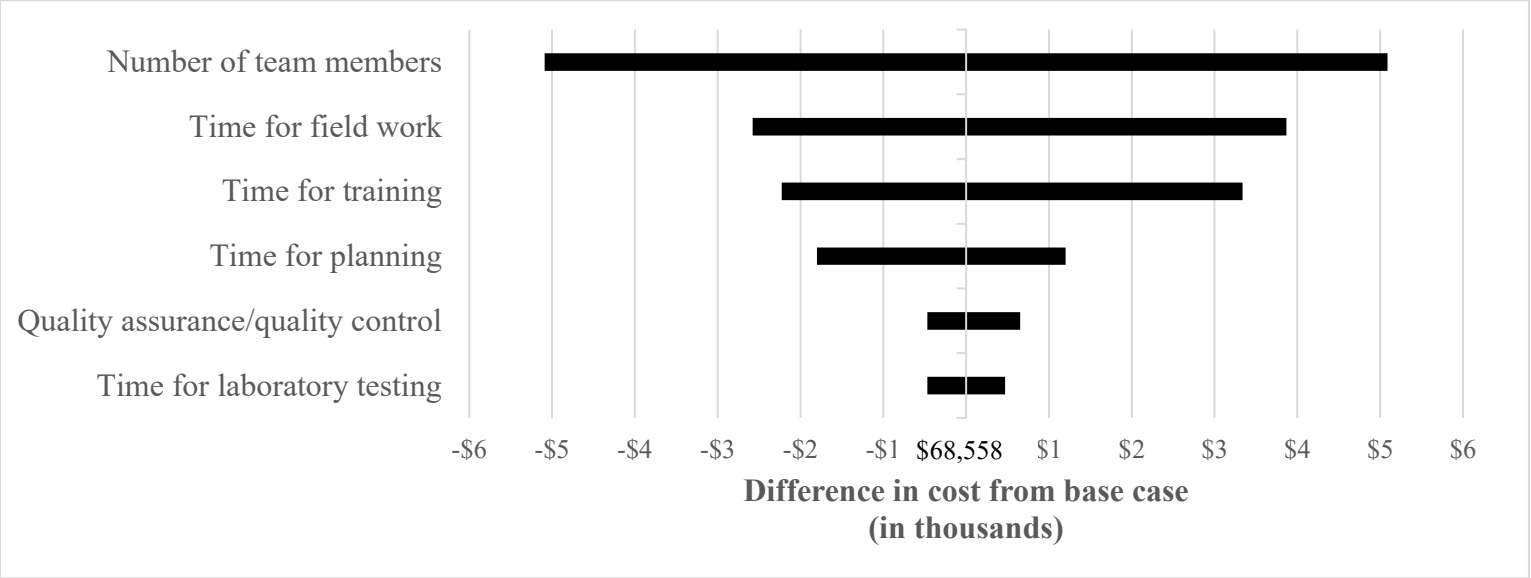
Notes: Framework was adapted from integrated disease surveillance and updated to capture serosurvey costs. Phase of study includes cores study activities. Columns represent input categories. Overlap across the matrix is captured in the costs.

Figure 8. Costs for the post-campaign vaccine coverage survey and serosurvey in Southern Province, Zambia by input category



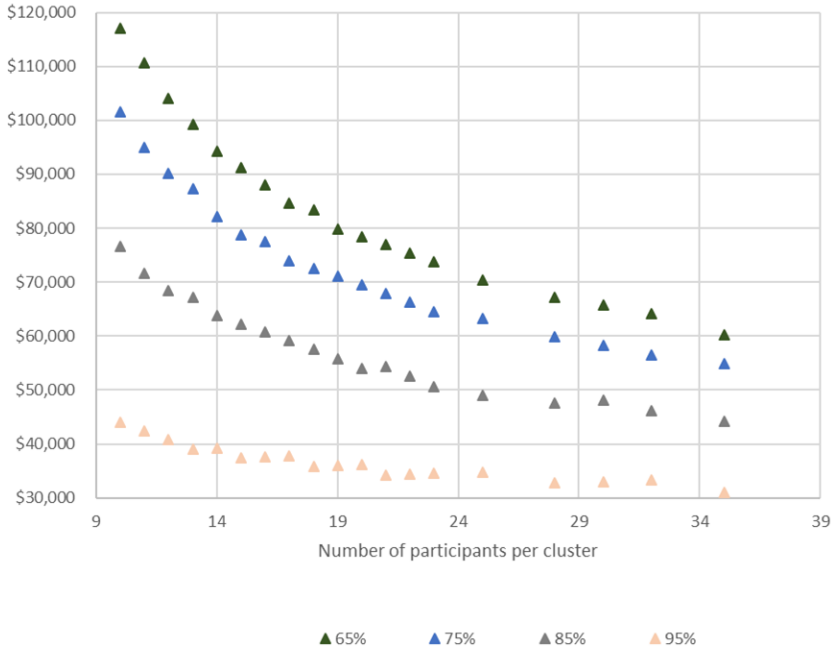
Notes: Costs captured for each input category span across serosurvey activities. Dark blue represents costs that would be inherent in a vaccination coverage survey, while light blue represents the costs that are specifically attributable to a serosurvey due to blood specimen collection, such as laboratory costs. All bars sum to the total cost. All costs are in 2016 USD.

Figure 9. One-way sensitivity analyses of costs varying serosurvey parameters



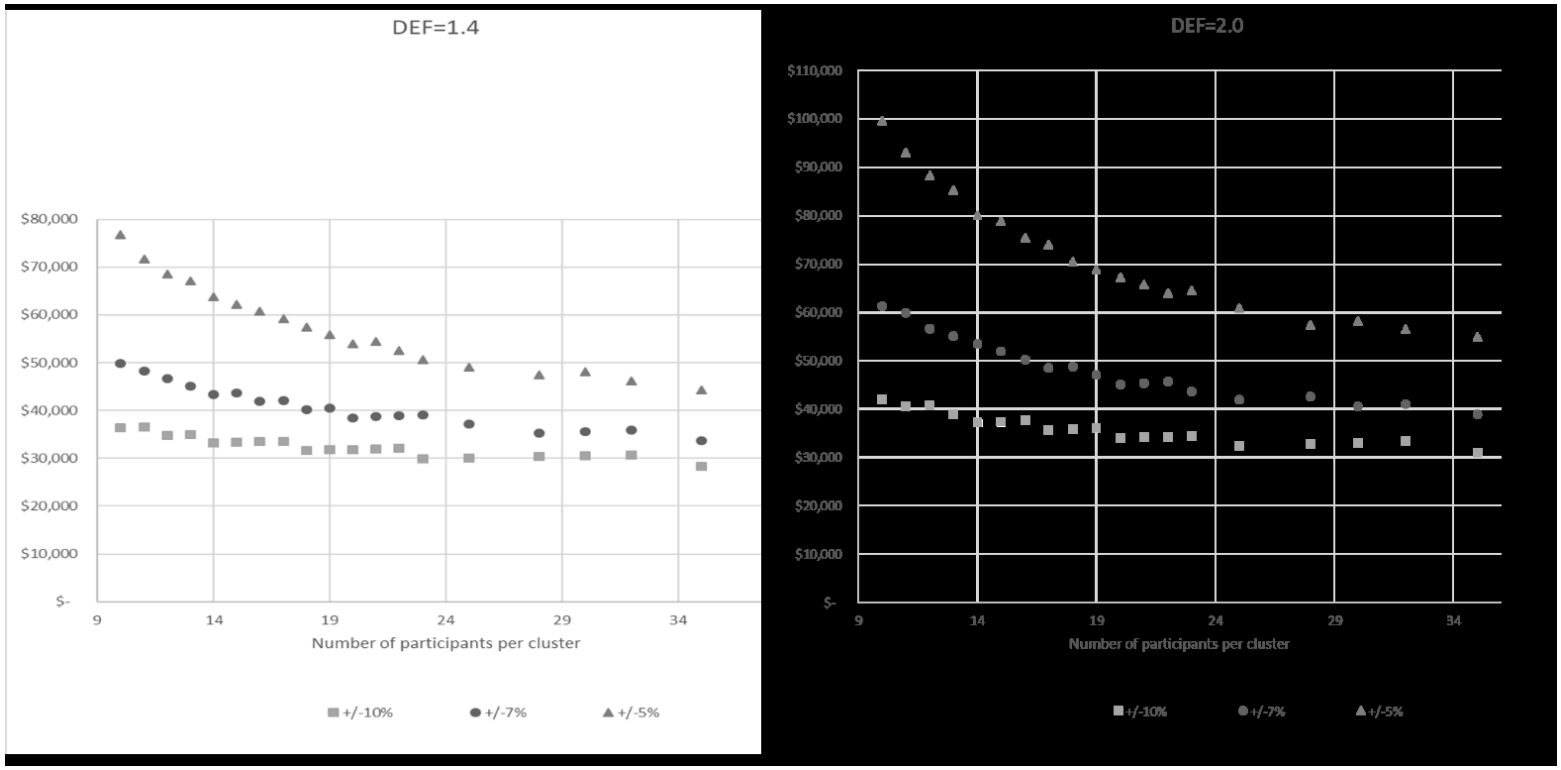
Notes: One-way sensitivity analysis represents how varying parameters increased (changed the total serosurvey cost from the base case of $68,558. Negative values (to the left) indicate lower cost than the base case, reflecting the lower end of the ranges, and the positive values (to the right) indicate higher cost than the base case, reflecting the higher end of the ranges. The number of team members varied from 2 to 6, the time for field work varied from 10 to 15 days, the time for training varied from 3 to 8 days, and the time for planning varied from 5 to 10 days. The percentage of specimens requiring retesting varied from 5 to 25% and the time for laboratory testing varied from 13 to 23 days. All costs are in 2016 USD.

Figure 10. Sample size costing with varying estimates of seroprevalence at +/-5% precision



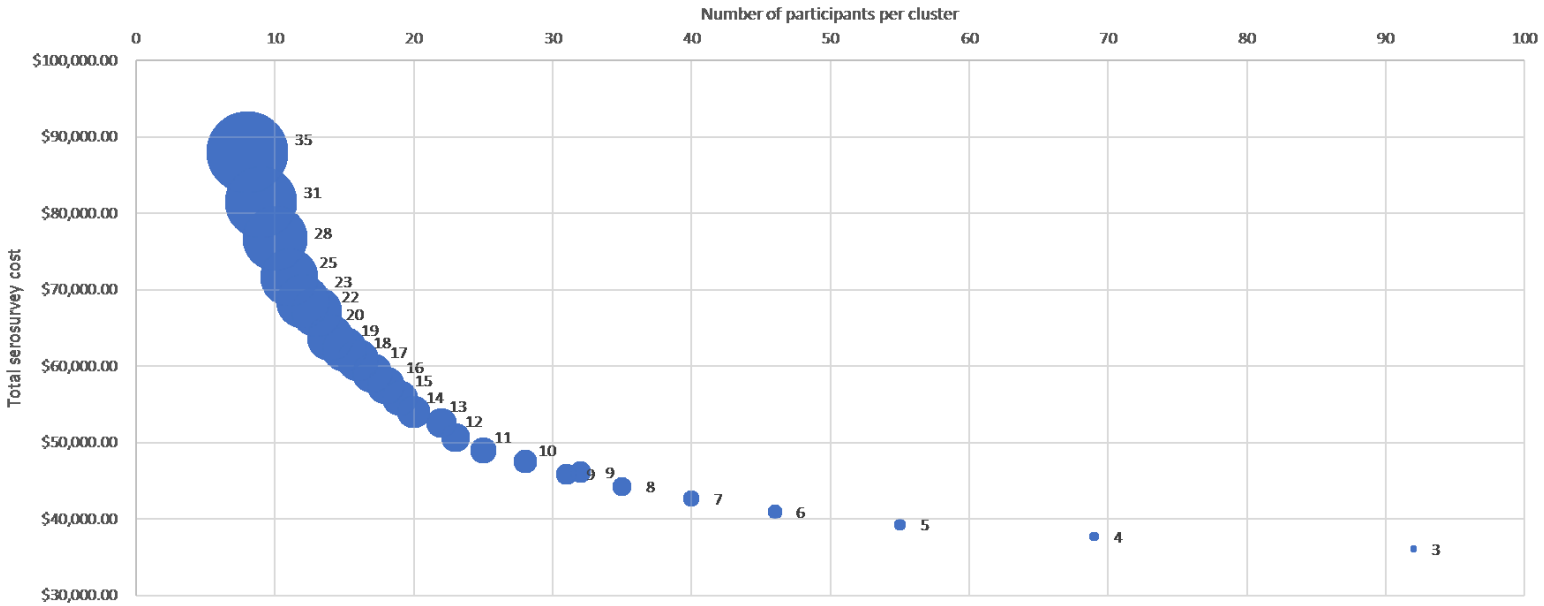
Notes: The number of participants per cluster, shown on the x-axis, determines the number of clusters required to maintain +/-5% precision (number of clusters not depicted). The design effect is assumed to be 1.4. Total cost= base cost ($22,960) + number of clusters*marginal cluster cost ($1,620) + number of participants per cluster *number of clusters* marginal cluster cost ($30). All costs in 2016 USD.

Figure 11. Sample size costing with varying precision at 85% seroprevalence with design effects of 1.4 and 2.0



Notes: Assumed estimated seroprevalence remains constant at 85%. The number of participants per cluster, shown on the x-axis, determines the number of clusters required to maintain +/-5% precision (number of clusters not depicted). The design effect is assumed to be 1.4 or 2.0. Total cost= base cost ($22,960) + number of clusters*marginal cluster cost ($1,620) + number of participants per cluster *number of clusters* marginal cluster cost ($30). All costs in 2016 USD.

Figure 12. Sample size options for setting with 85% population immunity and +/-5% precision



Notes: Assumed estimated seroprevalence remains constant at 85%. The number of participants per cluster, shown on the x-axis varies along with the number of clusters required to maintain +/-5% precision. Size of bubbles and number to the right of bubbles represent the number of clusters. The design effect is assumed to be 1.4, and alpha=0.05. Total cost= base cost ($22,960) + number of clusters*marginal cluster cost ($1,620) + number of participants per cluster *number of clusters* marginal cluster cost ($30). All costs in 2016 USD.

Paper 3- Comparing two serosurveys in Southern Province, Zambia to assess the impact of a measles and rubella vaccination campaign on seroprevalence

Abstract

Introduction: In September 2016, a national catch-up measles and rubella (MR) vaccination campaign was conducted in Zambia and was the first introduction of rubella-containing vaccine in the public sector. Given that approximately 95% population immunity is needed to interrupt measles virus transmission, a serosurvey can determine whether this threshold is met. To evaluate the impact of the vaccination campaign on population immunity, we compared the results of measles and rubella serosurveys conducted before and after the MR vaccination campaign to observe changes in seroprevalence in Southern Province, Zambia. Reported administrative first-dose measles coverage was 110% among children 12-24 months of age and second-dose measles coverage was 59% among children 24 months of age in 2016.

Methods: In early 2016, prior to the MR campaign, the Zambia Population HIV Impact Assessment was conducted as a cross-sectional household survey to estimate HIV incidence and prevalence. This study collected venous blood specimens from participants younger than 50 years of age. A sub-sample of 1,105 specimens from Southern Province were selected for measles and rubella testing based on HIV status, age, and cluster. In November 2016, a serosurvey to assess measles and rubella seroprevalence was conducted as part of a post-campaign vaccination coverage survey. This study collected dried blood spots from 543 participants 9 months to 49 years of age in 14 clusters. Specimens from these serosurveys were

tested for measles and rubella IgG antibodies using two commercially available enzyme-linked immunoassays (EIAs). Results were weighted to account for survey design, non-response, and adjusted for population age distribution. The two serosurveys were assessed for comparability.

Results: The overall seroprevalence among people under 50 years of age increased from 64.2% (95% CI: 59.3-68.8) before the MR vaccination campaign to 95.5% (95% CI: 93.6-97.5) after the campaign for measles, and rubella seroprevalence increased from 72.2% (95% CI: 67.6-76.3) to 95.9% (95% CI: 92.2-99.6). After the vaccination campaign, seroprevalence to measles increased from 57.2% (95% CI: 51.9-62.4) to 96.4% (95% CI: 91.7-98.5) among children younger than 15 years of age, and rubella seroprevalence increased from 51.3% (95% CI: 45.6-57.0) to 98.3% (95% CI: 95.5-99.4). In the pre-campaign serosurvey, age was associated with seroprevalence for both measles and rubella. Measles seropositivity was higher among HIV uninfected children under 15 years of age (57.5%, 95% CI: 52.1-62.7) than among HIV infected children (25.2%, 95%CI: 5.9-64.4). By contrast, seroprevalence to rubella was higher among HIV infected children under 15 years of age (100%) than among HIV uninfected children (50.9%, 95% CI: 45.1-56.6) in the pre-campaign serosurvey.

Conclusions: A comparison of the two serosurveys showed significant increases in seroprevalence from the pre-campaign to the post-campaign for both measles and rubella, highlighting the impact of the mass vaccination campaign in 2016. Despite high routine vaccination coverage, the pre-campaign survey found low measles seroprevalence in Southern Province, Zambia. The pre-campaign serosurvey also demonstrates differential seroprevalence rates among HIV infected children and the potential need for additional vaccination against measles. The serosurvey conducted after the MR vaccination campaign found high seroprevalence to both measles and rubella viruses, and there was no association of

seroprevalence with age, demonstrating how vaccination campaigns can close population immunity gaps.

Introduction

Measles is a highly infectious disease that was estimated to be one of the five major causes of death in children under 5 years of age in Zambia before the availability of vaccine (CDC, 2001). Thanks to measles vaccination, measles deaths dropped 84% from 550,100 in 2000 to 89,780 in 2016 worldwide, with Africa accounting for 44% of measles deaths (Dabbagh et al., 2018; World Health Organization, 2012).

Although not a major cause of childhood mortality, rubella is a major cause of congenital defects, estimated to affect 105,391 infants in 2010, with 37% of cases in Africa (Vynnycky et al., 2016). The most effective way to prevent congenital rubella syndrome (CRS) is through widespread immunization, ensuring that women of childbearing age are protected against rubella.

The African Region established a goal of measles elimination by the end of 2020. The primary component of elimination strategy is to achieve high population immunity by providing high vaccination coverage with two doses of measles and rubella-containing vaccines (World Health Organization, 2012).

Measles vaccination was introduced into the routine immunization program in Zambia in 1983; however, widespread outbreaks continued with 26,072 suspected cases of measles reported annually from 1994-2003 (CDC, 2005). To improve population immunity to measles virus, vaccination campaigns were conducted in 2003, 2007, 2010, and 2012. However, there was still a nationwide measles outbreak in 2010-2011 with up to 37,582 cases reported (Mpabalwani ME,

2013). Zambia introduced a second dose of measles vaccine (MCV2) in 2013, but coverage was only 56% in 2016 (Zambian Ministry of Health, 2019b).

In September 2016, a national catch-up measles and rubella (MR) vaccination campaign was conducted in Zambia targeting all children 9 months to younger than 15 years of age. This campaign was the first introduction of rubella-containing vaccine (RCV) in the public sector, and RCV was included in the routine immunization program (Masresha et al., 2017).

The impact of vaccination campaigns can be assessed in multiple ways, including vaccination coverage achieved by the campaign, the number of zero-dose children vaccinated, and the proportion of children receiving at least one or two vaccine doses. However, these do not account for potential vaccine failures. Tracking the number of cases reported before and after a campaign can also be used to evaluate campaign effectiveness if surveillance is sufficiently sensitive to identify cases. Zambia's fever rash surveillance struggles to meet targets for indicators of a sensitive surveillance system. Monitoring cases is also a lagging indicator, as it identifies population immunity gaps that existed at time of measles virus transmission.

Serological surveillance can provide a more direct measurement of changes in population immunity before and after a vaccination campaign. Given that approximately 95% population immunity is needed to interrupt measles virus transmission, a serosurvey can determine whether this threshold is met. To evaluate the impact of the vaccination campaign on population immunity, we compared serosurveys conducted in Southern Province, Zambia before and after the 2016 MR vaccination campaign to observe changes in population immunity. To determine the comparability of the two serosurveys, we evaluated the impact of different sampling strategies, specimen types, and laboratory assays on the seroprevalence estimates generated from the serosurveys.

Methods

Study setting

This study was conducted in Southern Province, the third most populous province in Zambia with 1.9 million inhabitants across 13 districts in 2016 (Zambia Central Statistical Office, 2013). The population age structure is expanding with 48% of the population under 15 years of age and a total fertility rate of 5.7. The infant mortality rate remains high at 62 per 1,000 live births, and life expectancy at birth is 59 years of age. Southern Province has the fourth highest HIV prevalence in Zambia, estimated at 13% in 2016 (Columbia University, 2016). Reported administrative first-dose measles coverage was 110% among children 12-24 months of age and second-dose measles coverage was 59% among children 24 months of age in 2016 (Zambian Ministry of Health, 2019b).

Data and biospecimen collection

Specimens were collected within two studies conducted in Southern Province in 2016. The first study collected specimens from participants before the MR vaccination campaign, and the second collected specimens 3 months after the campaign. Table 12 compares the study designs for these two serosurveys.

All studies were approved by the Institutional Review Board at Johns Hopkins Bloomberg School of Public Health and the Zambian Ministry of Health. The pre-vaccination campaign was approved by the National Regulatory Authority, and the post-vaccination campaign was approved by Macha Research Trust.

Pre-vaccination campaign data collection

In early 2016, the Zambia Population HIV Impact Assessment (ZamPHIA), was conducted as a cross-sectional community survey to estimate HIV incidence and prevalence. The survey design was a two-stage cluster survey methodology with clusters selected from the 2010 census with probability of selection proportional to estimated size. Within each cluster, households were listed, and a random sample of households within each cluster were selected based on a computer algorithm. This study then collected survey data and venous blood specimens from adults 15 to 49 years of age in every household selected. In half of the households selected, children younger than 15 years of age were also included, with dried blood spots collected from finger prick for children 6-23 months of age and from heel prick for children under 6 months of age (Ministry of Health, Zambia, 2017). This study was powered to estimate provincial HIV incidence rates by selecting enumeration areas in each province. This study reported 11% households refused to provide blood specimens, and only 68% of eligible children participated (Columbia University, 2016). HIV home-based testing and counseling were conducted. Individuals with nonreactive result on the screening test were classified as HIV uninfected. Those with a reactive test result underwent confirmatory testing using Uni-Gold, and if reactive again were classified as HIV infected. Individuals with reactive screening and nonreactive confirmatory tests were classified as indeterminate and counseled to seek a repeat test in 4 weeks, according to national guidelines (Columbia University, 2016). After specimens were transported to the central laboratory and completed any additional HIV testing, residual plasma and dried blood spots study were frozen in a biorepository at -70°C in Ndola, Zambia.

Residual plasma and DBS samples from this study were tested for anti-measles virus and anti-rubella virus IgG antibodies. Of the 2,648 specimens collected in Southern Province, 1,105

were selected for testing to estimate age-specific seroprevalence to measles and rubella with 10% precision. Specimens were selected based on HIV status, cluster representation, and age. All HIV infected participants and clusters with fewer than 10 participants were selected for testing. To ensure sufficient sample size for age-specific seroprevalence estimates, participants <5, 5-9, and 10-14 years of age were selected to represent 22% of the subsample in each category, and participants 15-19 and 20-49 years were selected to represent 17% of the subsample in each category. Additionally, at least one respondent from every cluster was included. Serological results were linked to clinical and demographic variables in the ZamPHIA database but data on measles vaccination status were not collected.

Post-vaccination campaign data collection

In November 2016, a serosurvey to assess measles and rubella seroprevalence was conducted as part of a post-campaign vaccination coverage survey conducted by the Ministry of Health. The nested serological survey used the same sampling strategy as the post-campaign vaccination coverage survey, a two-stage cluster survey with the clusters selected from the 2010 census with probability of selection proportional to estimated size. Twenty-six clusters were selected in Southern Province. Within each cluster, households with children in the post-campaign vaccination coverage survey target age group (9 months to less than 15 years) were eligible for enrollment. Households in each cluster were listed and designated as either (1) having all children vaccinated or (2) having at least one child unvaccinated. A total of 12 households per cluster were enrolled, with a stratified allocation ratio of 2:1 vaccinated to unvaccinated households to permit analysis of reasons for not being vaccinated. If there were not sufficient unvaccinated households, vaccinated households were included instead.

The nested serosurvey was designed to estimate seroprevalence within +/-7% for prespecified age groups (<5, 5-9, 10-14, and 15 years of age and older). This serosurvey was nested in 14 of the 26 clusters selected for Southern Province. All households included in the vaccination coverage survey were eligible for the nested serosurvey. Whereas the vaccination coverage survey included only children eligible for the campaign, the nested serosurvey included all members 9 months of age and older who were present at the time of the serosurvey. Dried blood spots were collected by fingerpick from all participants, placed in plastic storage bags with a desiccant, and kept at room temperature for 1-3 days until transfer to the laboratory for long-term storage at -20° C (Hayford et al., 2019). Information on measles vaccination history, including routine measles vaccination and MR vaccination during the campaign, were collected for children younger than 15 years of age. HIV infection status was not known for these participants.

Laboratory methods

For the pre-campaign serosurvey, plasma specimens were thawed overnight at 4°C. Specimens were processed as recommended by the manufacturer and tested for anti-measles virus IgG and anti-rubella virus IgG antibodies using indirect enzyme immunoassays (Euroimmun; Lübeck, Germany). Blood collected as DBS was eluted according to a protocol optimized for use with the Euroimmun assays. A quantitative antibody concentration was generated based on curves and manufacturer's thresholds. Measles results were classified as positive (≥275 mIU/mL), equivocal (≥200 to <275 mIU/mL), or negative (<200 mIU/mL). Rubella results were classified as positive (≥11 IU/mL), equivocal (≥8 to <11 IU/mL), or negative (<8 IU/mL). Antibody concentrations for rubella were left-censored at the lower level

of detection (0.2 IU/mL) and right-censored at the upper level of detection (assigned 5,001 IU/L for measles and 201 IU/mL for rubella). Values above the upper limit of detection were not diluted and retested. Equivocal results were retested and, if equivocal again, were categorized as positive for analyses. Quality control and quality assurance procedures are detailed in Appendix 5. A subset of 88 participants that had both DBS and plasma specimens were tested to assess concordance between DBS and plasma. Since these found 85% qualitative concordance for measles and 100% for rubella, we opted not to adjust the DBS specimens. Testing was split between the National Virology Lab at the University Teaching Hospital in Lusaka and the Tropical Disease Research Center in Ndola. Testing was conducted from April to October 2019 with near-real time data management and analysis from Johns Hopkins University.

For the post-campaign serosurvey, serum eluted from DBS were tested for IgG antibodies to measles and rubella viruses with indirect enzyme immunoassays (Enzygnost; Siemens, Munich, Germany) at Macha Research Trust. The circumference of each DBS was measured, cut with sterilized scissors, and serum was eluted with 250 µL of buffer as described in an active elution protocol developed at the Centers for Disease Control and Prevention (Mercader, Featherstone, & Bellini, 2006). Fifty microliters of eluted sample were transferred to precoated 96-well plates and the manufacturer's protocol was followed to perform the EIA. The optical density (OD) was read at 450 nm using a BioTek ELx800 microplate reader (BioTek Instruments, Winooski, VT). Corrected optical density (cOD) differences were calculated based on differences in OD values for the antigen and control wells, the mean of positive and negative controls, and lot-specific parameters, as specified by the manufacturer. Specimens were classified as positive (>0.2 cOD), equivocal (0.1 – 0.2 cOD), or negative (<0.1 cOD); this would be approximately equal to 150 mIU/mL as the upper cut-off for negatives. For samples with cOD

≥0.1 and OD≤2.5, measles (mIU/mL) and rubella (IU/mL) antibody concentrations were calculated according to the manufacturer protocol. Equivocal results were re-tested and, if equivocal again, were categorized as positive for analyses. Adjustments were made to the DBS results to account for volume, spot size and diagnostic accuracy of DBS compared to a subset of serum (Hayford et al., 2018).

Euroimmun reports 100% sensitivity and 100% specificity for measles IgG antibodies and Siemens reports 99.6% sensitivity and 100% specificity in the product inserts. Euroimmun reports 99.6% sensitivity and 100% specificity to rubella IgG, compared to Siemens having 100% sensitivity and 98.5% specificity. A head-to-head comparison of Euroimmun and Siemens rubella kits found 96.3% agreement for IgG in plasma (Hardelid et al., 2008). There is no reported head-to-head comparison for the measles kits.

Data analysis

Results were weighted to account for the inverse probability of selection, non-response, and post-stratification adjustments based on each survey design. The pre-campaign serosurvey used individual-level weights based on selection probability (by age, HIV status, and cluster), non-response based on non-availability of specimens in the biorepository, and post-stratification adjustment to the Southern Province population age and sex structure. For the post-campaign serosurvey, household-level weights were calculated based on selection probability by vaccination status and cluster, non-response based on number of households that refused to participate despite being included in the household listing in the clusters, and post-stratification to account for any changes in the sampling frame predictions from the 2010 census predictions (Zambian Ministry of Health, 2017). Given the differential selection criteria, Figure 13 depicts

factors associated with inclusion in each serosurvey and how they were adjusted to calculate the overall seroprevalence estimates.

Categorical variables and age-specific seroprevalence estimates were compared using Rao-Scott chi-square tests. Penalized regression spline was used to model age-specific seroprevalence. Overall provincial seroprevalence estimates were calculated using direct age and sex adjustment based on 2016 population estimates from Zambian Central Statistics Office. This involved applying the proportion seropositive in each age and sex category to the standard population estimates from Southern Province to provide the expected number of seropositive people for each age and sex group based on a common population distribution. Summing the total number of seropositive people in the standard population provides the adjusted provincial seroprevalence (Tripepi, Jager, Dekker, & Zoccali, 2010).

Results

From the pre-campaign serosurvey, 1,105 specimens were tested for measles and rubella IgG antibodies, and 543 specimens were tested from the post-campaign serosurvey. The pre-campaign serosurvey had more adults 15 years of age and older (49%) than the post survey (34%) (Table 13). Additionally, males were under-represented in the post-campaign serosurvey (45%). In the oldest age group (20-49 years), 49% of participants were male in the pre-campaign serosurvey, whereas only 25% were male in the post-campaign serosurvey. In the pre-campaign serosurvey, 7% of participants were HIV seropositive.

Seroprevalence by age

Measles seroprevalence prior to the vaccination campaign was 57.2% (95% CI: 51.9-62.4) and increased to 96.4% (95% CI: 91.7-98.5) among participants younger than 15 years of age, those targeted during the campaign. Surprisingly, measles seroprevalence was higher among adults in the post-campaign serosurvey (93.3%, 95%CI: 84.5-97.3) compared to the pre-campaign serosurvey (71.5%, 95% CI: 63.8-78.1). Figure 14 depicts the close in the seroprevalence gap from pre to post-campaign serosurvey by age among those under 30 years of age. Rubella seroprevalence increased from 51.3% (95%CI: 45.6-57.0) to 98.3% (95%CI: 95.5-99.4) among children younger than 15 years of age after the vaccination campaign. Figure 12 depicts the close in the seroprevalence gap from pre to post-campaign serosurvey by age among those under 15 years of age. Unlike measles, there was no increase in rubella seroprevalence among adult age groups after the MR vaccination campaign.

While there was some variability in measles seroprevalence by age group, there were no statistically significant differences within either the pre or post-campaign serosurvey (Figure 15). By contrast, in the pre-vaccination campaign serosurvey rubella seroprevalence had a strong positive association with age (Figure 15). Rubella seroprevalence significantly increased in each age category in the pre-campaign serosurvey, from 23.9% (95% CI: 17.8-31.2) among children under five-years old to 96.2% (95% CI: 93.0-98.0) in adults 20-49 years of age.

Seroprevalence by sex

Given the selection bias by sex, we conducted a stratified analysis of seroprevalence by sex to see if this led to differences in seroprevalence. Although there were no statistically significant differences, measles seroprevalence in the pre-vaccination serosurvey was slightly

higher in females (65.8%, 95%CI: 58.8-72.1) than males (62.6%, 95%CI: 57.8-67.1) for measles, and rubella seroprevalence was higher in females (73.6%; 95%CI: 67.2-79.2) than males (70.7%; 95%CI: 65.8-75.2).

Similarly, we conducted a stratified analysis of seroprevalence by sex within age categories to see if there were differences. Although there were no statistically significant differences in sex, like the overall sex differences in the pre-campaign serosurvey, females had slightly higher measles seroprevalence than males in almost every age category (Figure 16). Similarly, females had slightly higher rubella seroprevalence among children under 10 years of age and adults 20-49 years old. (Figure 17).

Differences in antibody concentrations

Geometric mean concentration for measles increased among children under 15 years of age from 270 mIU/mL (95% CI: 254-287) before the campaign to 1642 mIU/mL (95% CI: 1507-1788) after the campaign (Figure 18). The distribution of measles antibody concentrations shifts from a tight distribution peaking around the 200 mIU/mL cutoff in the pre-campaign serosurvey to a disperse distribution in the post serosurvey. The under 15-year-old participants shift to a peak around 2000 mIU/mL, consistent with seroconversion as well as boosting following vaccination. Among adults 15 years of age and older, geometric mean concentrations were 475 mIU/mL (95% CI: 421-536) in the pre-campaign serosurvey and 1554 mIU/mL (95% CI: 1344-1795) in the post-campaign serosurvey. Again, the distribution peak shifts from being around 200 mIU/mL in the pre-campaign serosurvey to around 1,000 mIU/mL in the post-campaign serosurvey. Overall, 1.5% of specimens in the pre-campaign serosurvey and 8.3% of specimens

in the post-campaign serosurvey were above the upper limit of detection for measles. No measles specimens were below the lower limit of detection (8 mIU/mL).

Geometric mean concentration for rubella increased among children under 15 years of age from 14 IU/mL (95% CI: 11-17) before the campaign to 153 IU/mL (95% CI: 145-161) after the campaign. Rubella antibody concentrations shifted from a bimodal distribution in the pre-campaign serosurvey for those under 15 years of age, to skewed single peak at the upper limit of detection (200 IU/mL) in the post-campaign serosurvey (Figure 18). Among adults 15 years of age and older, geometric mean concentrations were 85 IU/mL (95% CI: 72-101) in the pre-campaign serosurvey and 132 IU/mL (IQR: 120-145) in the post-campaign serosurvey. The distribution for adults 15 years of age and older are similar in the pre and post-campaign serosurveys, with a peak at the upper limit of detection. Overall, 46.4% of specimens in the pre-campaign serosurvey and 52.1% of post-campaign specimens were above the upper limit of detection for rubella (200 IU/mL). In the pre-campaign, 12% of rubella specimens were below the lower limit of detection (0.3 IU/mL), and there were none in the post-campaign.

Seroprevalence by HIV status in the pre-campaign serosurvey

The pre-campaign serosurvey tested participants for HIV; therefore, we stratified seroprevalence by HIV infection status to see the effect of HIV on seroprevalence (Figure 19). Adults 15 years of age and older had higher HIV prevalence (13.8%, 95% CI: 8.8-20.9) than children under 15 years of age (0.9%, 95% CI: 0.5-1.7). Measles seropositivity was higher among HIV uninfected children under 15 years of age (57.5%, 95% CI: 52.1-62.7) than among HIV infected children (25.2%, 95%CI: 5.9-64.4). There were no differences in measles seropositivity by HIV infection status among adults 15 years of age and older.

On the other hand, rubella seroprevalence was significantly higher among HIV infected children under 15 years of age (100%) than among HIV uninfected children (50.9%, 95% CI: 45.1-56.6) (Figure 19). The difference in rubella seropositivity by HIV infection status is no longer statistically significant among adults 15 years of age and older.

Overall provincial seroprevalence estimates and effects of weighting and adjustments

The overall seroprevalence for measles increased from 64.2% (95% CI: 59.3-68.8) before the MR vaccination campaign to 95.5% (95% CI: 93.6-97.5) after the campaign, after accounting for serosurvey designs and adjusting for different age and sex distributions in the serosurveys. Rubella seroprevalence increased from 72.2% (95% CI: 67.6-76.3) to 95.9% (95% CI: 92.2-99.6) after the MR vaccination campaign. Weighting by survey design tended to increase seroprevalence in the pre-campaign but did not substantially affect post-campaign seroprevalence (Table 14). Excluding HIV infected individuals from the pre-campaign serosurvey also did not change seroprevalence estimates. While all provincial estimates were similar regardless of weighting and adjustment, the weighted and age and sex adjusted estimates represent the most comparable populations.

Discussion

Measles and rubella seroprevalence significantly increased after the MR vaccination campaign in the target age children in Southern Province, Zambia. To determine whether this was a true increase in seroprevalence, it was first necessary to address the comparability of the two serosurveys. Seroprevalence to measles was lower than expected in the pre-campaign serosurvey in Southern Province, as they have reported high first-dose measles coverage for

many years, with 11 of 13 districts reporting coverage >95% in 2016, and introduced a second-dose of measles vaccine in 2013 (Zambian Ministry of Health, 2019b). Participants 18 to 54 months of age would have been eligible to receive 2 doses of routine measles containing vaccine, and participants 5-30 years of age would have had opportunities for vaccination during at least two past vaccination campaigns. In contrast, rubella seroprevalence from the pre-campaign serosurvey followed a typical age-specific seroprevalence curve prior to the introduction of rubella vaccine (Edmunds et al., 2000; Gomwalk & Ahmad, 1989; Winter et al., 2018). The serosurvey conducted after the vaccination campaign identified high seroprevalence for both measles and rubella.

Differences in sampling and probability of inclusion

These data were collected from two different cross-sectional studies that both had sub-sampling within main studies and had different survey designs. However, both main studies used the same sampling frame of the 2010 census for Southern Province and selected clusters in the first stage of sampling based on probability proportional to size. At the second and third levels of sampling, the primary studies differed. The pre-campaign main study randomly selected households whereas the post-campaign serosurvey stratified household based on vaccination status. If more households with unvaccinated children were included than vaccinated, this could have artificially decreased seroprevalence in the post-campaign serosurvey, but this was accounted for in the selection probability weighting. The pre-campaign main study included adults 15 years of age and older in each household, but only children under 15 years of age in every other household. If the households with children included were less likely to be seropositive than those with children who were not included, this could bias the seroprevalence

estimate downwards. By contrast, the post-campaign main study focused on enrolling children 15 years of age or younger.

In terms of the sub-sampling from each of the main studies, the pre-campaign serosurvey maintained all clusters in the main study whereas the post-campaign serosurvey randomly selected 14 of 26 clusters. At the individual level, the pre-campaign serosurvey sub-sampled based on HIV status and age; whereas the post-campaign serosurvey included all participants present in the household at the time of data collection. At each of these levels, there could have been selection bias. To account for differential probabilities of inclusion, both serosurveys calculated probability of selection weights. These were multiplied by non-response weights to account for differential response rates. Weighting both serosurveys should have made them comparable to the original population of Southern Province used for the sampling frame.

Differences in population distributions

Both serosurveys included individuals 0-49 years of age; however, there were differential age distributions between the serosurveys. There were more older adults 20-49 years old included in the pre-campaign serosurvey than in the post-campaign serosurvey. Given the strong association between age and seroprevalence for both measles and rubella, older participants would be more likely to be seropositive. Therefore, comparing overall seroprevalence from the pre and post-campaign serosurveys can only be done if they are age-adjusted to a standard population.

The increase in seroprevalence for children under 15 years of age was expected due to the vaccination campaign reaching 96.8% coverage (95% CI: 94.7-98.1) (Zambian Ministry of Health, 2017). In the 15-19-year age group, there was a slight spillover effect with six participant

15 years of age at the time of the campaign reporting having been vaccinated during the campaign. However, for adults 20-49 years, the reasons for an increase in measles seroprevalence after the MR vaccination campaign remain unclear. While this could have been due to measles outbreaks, only two cases of laboratory-confirmed measles were reported in 2015-2016, and measles virus transmission typically peaks October-December when the post-campaign serosurvey was conducted (Zambian Ministry of Health, 2019a). Alternatively, the increase in seroprevalence could be due to selection bias in the serosurveys based on the differential age distribution. To address this difference, seroprevalence was directly age-adjusted to the 2016 population for Southern Province; however, the increase in measles seroprevalence among adults 20-49 years remained.

Although there were differences in the proportion of females and males between the two serosurveys, there were not significant differences in seroprevalence by sex. While there may be different immunological responses by sex to vaccination for measles and rubella, it is not clear that these would make one more likely to be seropositive (Klein, Marriott, & Fish, 2015; Mitchell, 1999). We addressed the differences in sex between the two serosurveys through direct adjustments within each age category, based on the 2016 population for Southern Province. This should have made the two serosurvey populations comparable. These adjustments did not substantially affect the overall seroprevalence point estimates.

Potential confounders

Another factor that could threaten the comparability between the pre- and post-MR campaign serosurveys is confounding by other characteristics, such as HIV infection status. A recent review did not find population-level differences in seroprevalence to measles by HIV

infection status for adolescents and adults (Loevinsohn, Rosman, & Moss, 2019). However, reviews of studies in children found lower seroprevalence to measles among HIV infected compared to HIV uninfected children (Mehtani, Rosman, & Moss, 2019; Scott, Moss, Gilani, & Low, 2011). A study conducted in our study setting of Southern Province found that HIV infected children tended to have lower measles seroprevalence (Sutcliffe et al., 2017). Similarly, our estimates showed HIV infection was associated with a lower measles seroprevalence in children. If we captured fewer HIV-infected children in the post-campaign serosurvey than are present in the population, this would have inflated our estimate of measles seroprevalence. This could have occurred if HIV-infected participants were less likely to participate in the serosurvey (Welz et al., 2007). However, there was no difference in participation rates between the two serosurveys.

Our findings also demonstrated HIV-infected participants were more likely to be seropositive to rubella. Rubella virus circulation was common before vaccine was introduced, with 15 laboratory-confirmed cases in 2015-2016, and 80% of individuals being infected by the age of 10 years. Individuals infected with rubella prior to HIV infection have been shown to have higher seroprevalence to rubella than age and sex-matched HIV uninfected counterparts (Thomas & Aird, 1999). However, we had a small sample size of HIV infected children, none who were under 5 years of age and only 6 who were 5-9 years of age. Given we did not test for HIV status in the post-campaign serosurvey, it was not possible to confirm whether there was a difference in HIV infection status between the two serosurveys. However, sensitivity analyses excluding HIV-infected participants from the pre-campaign serosurvey did not change the population estimate or the difference between pre and post-campaign serosurveys.

Differences in specimen collection type

Another potential explanation for differences in the serosurvey results is specimen type. Plasma specimens were collected in the pre-campaign serosurvey, while DBS were collected in the post-campaign serosurvey. In the pre-campaign serosurvey, plasma specimens were frozen for 2 years prior to testing. However, specimens underwent only one freeze-thaw cycle and thus should not have degraded (World Health Organization, 2019). The pre-campaign serosurvey also demonstrated high concordance between DBS and serum, as has been reported previously for measles and rubella IgG (93%) (Helfand et al., 2001). The post-campaign serosurvey also collected a sub-set of serum for 200 participants, which allowed DBS results to be adjusted to account for the use of DBS rather than sera for the assay kits (Hayford et al., 2019). Accounting for differential specimen type through these adjustments and subsamples should have made the serosurveys comparable in terms of biospecimens collected.

Differences in enzyme immunoassay kits

An additional consideration for differences in the serosurvey results was the use of different EIA kits. EIAs risk misclassification bias due to predefined cut-off values being designed for individual patient management rather than population antibody level identification, and thus they tend to err towards having higher thresholds for seropositivity (World Health Organization, 2015). The high proportion of participants in the pre-campaign centered around 200 mIU/mL suggest that if the cut-off were too high, this could have resulted in lower observed seroprevalence than the true value. Additionally, different EIA kits were used in the two serosurveys: the pre-campaign serosurvey used Euroimmun whereas the post-campaign serosurvey used Siemens Enzygnost EIA kits. Both kits report similar sensitivity and specificity.

The similar quantitative rubella antibody concentration distribution among adults illustrates the expected similar seroprevalence among adults measured by both EIA kits. The measles antibody concentration range among adults were similar before and after the campaign for adults; however, the shift from low-range concentration to high-range concentration was unexpected.

Limitations

Although several key aspects of the differences between the two serosurveys were accounted for through weighting, direct adjustments, and head to head comparison of testing, there was still one unexpected result in the findings. The increase in seroprevalence to measles among adults 20-49 years old could not be explained with the available data. This could represent a difference in who elected to participate in the serosurvey. Although, these two serosurveys were conducted in the same area within a one-year period, we do not believe this affected participation, given similar refusal rates in both studies. If this was due to differential seropositivity due to HIV infection status, we could not adjust the post-campaign serosurvey, as these data were not available. Similarly, we cannot rule out whether this was due to a difference in the sensitivity of the measles EIA kits. A lower sensitivity to measles seropositivity in the Euroimmun kit has been previously reported ((Tischer et al., 2007). A comparison of the measles IgG kits was not possible, as Siemens stopped manufacturing the kit in 2017.

Conclusion

Two cross-sectional serosurveys were able to demonstrate significant increases in seroprevalence from the pre-campaign to the post-campaign for both measles and rubella, highlighting the impact of the mass vaccination campaign in 2016. Weighting for survey design

selection probability and non-response and direct adjustments to a common age and sex population distribution were done to probe if the observed differences in seroprevalence were real. While the pre and post-campaign serosurveys had many differences in study design, these were mostly addressed to make the serosurveys comparable.

When serosurveys are collected in separate studies, consideration of the different objectives, study design, implementation and laboratory testing should be made to compare results. Understanding the variables that could be associated with seropositivity and ensuring these are measured could help improve comparability of results. Similarly, collecting sub-samples of different specimen types and performing head-to-head comparisons if different laboratory tests are used can help compare the results of serosurveys.

Tables and Figures

Table 12. Comparisons between pre and post-campaign serosurvey design

	ZamPHIA (pre campaign)	**Nested serosurvey (post campaign)**	**Potential for selection bias**
Number of clusters	55	14	
Number of households	529	133	
Districts covered in Southern Province	Choma Gwembe Itezhi-tezhi Kalomo Kazungula Livingstone Mazabuka Monze Namwala Siavonga Sinazongwe	Choma Gwembe Kalomo Kazungula Livingstone Monze Namwala Pemba	
Methods—sampling of main study	<u>First stage</u> Clusters based on probability proportional to size	Clusters based on probability proportional to size	
	<u>Second stage</u> Households approximately based on number needed for HIV incidence estimation, approximately 30 per cluster	Household based on campaign vaccination status of children, 12 per cluster	In post serosurvey, if there were more unvaccinated households included, could lower seroprevalence estimate

	Third stage Children <15 years old in every other household, adults ≥15years old in every household	Children eligible for the campaign (9 months to <15 years old)	In pre serosurvey, if households with children included were more likely to be vaccinated, could increase seroprevalence
Methods—sampling of sub-study	First stage All clusters represented	14 of 26 clusters	In post serosurvey, if included clusters were more likely to be vaccinated, could increase seroprevalence
	Second stage Not considered	All households included in the selected clusters	
	Third stage All HIV infected individuals; Age-specific probabilities (22% 0-4, 5-9, 10-14-year olds and 17% 15-19, 20-49-year olds)	All household members home at the time of the survey in selected households	In pre serosurvey, if HIV infected were less likely to be seropositive, could decrease seroprevalence In post serosurvey, if those present at time of serosurvey were more likely to be seropositive, could increase seroprevalence
Biospecimen collection	Plasma collected from venipuncture for participants ≥2 years of age; DBS from finger prick for participants 6-	Dried blood spot collected from fingerpick from all participants	In post serosurvey, if DBS had higher antibody concentration, could increase seroprevalence*

	23 months and heel prick for under 6 months		
Laboratory process	EUROIMMUN ELISA tests	Siemens ELISA tests	In post serosurvey, if Siemens tests are more sensitive and have lower cutoff, could increase seroprevalence*
Timing	Months before vaccination campaign	3 months post vaccination campaign	
Refusal rates	11% of individuals participated in the survey but refused blood collection	12% of individuals at enrolled households refused to participate in the serosurvey	In post serosurvey, if participants had already participated in one serosurvey may refuse second one

Notes: Table compares methodologies between pre and post serosurveys. Selection bias column indicates which serosurvey could be biased based on serosurvey design. All biases are selection bias unless indicated by (*), which indicate potential laboratory biases.

Table 13. Study population characteristics in pre and post-campaign serosurveys, weighted

	Pre-campaign (n=1,111)	**Post-campaign** (n=590)	**P-value**
Age (%)			<0.001
0-4	20.0	21.7	
5-9	17.4	26.9	
10-14	13.7	17.3	
15-19	12.0	7.4	
20-49	36.9	26.7	
Male (%)	49.8	44.5	0.06
Males by age (%)			<0.001
0-4	50.6	56.0	
5-9	50.5	48.5	
10-14	50.5	53.1	
15-19	50.2	46.3	
20-49	48.7	25.2	
Number of people per cluster (n(SE))	24 (1.2)	44 (3.1)	<0.001
Number of people per household (n(SE))	3 (0.1)	5 (0.3)	<0.001

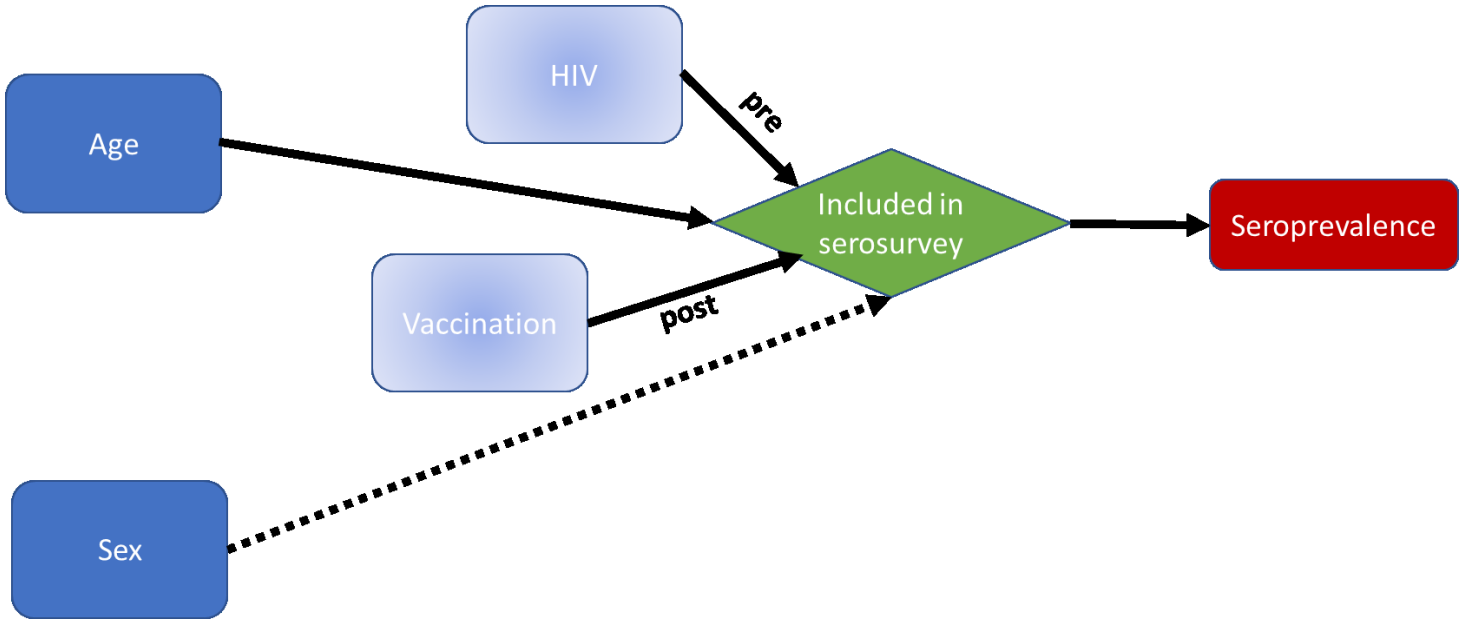
Notes: Characteristics presented as weighted percentages to assess if differences remain after accounting for serosurvey design, as designated by the p-value.

Table 14. Provincial level seroprevalence estimates to measles and rubella pre and post-vaccination campaign—unweighted, weighted, and direct adjustments

	Measles Pre	**Measles Post**	**Rubella Pre**	**Rubella Post**
Unweighted	59.9%	95.6%	68.5%	97.3%
Weighted based on survey design	63.3% (62.2-64.3)	95.5% (92.8-97.2)	66.6% (65.4-67.7%)	97.7 (96.0-98.7)
Direct age adjustment unweighted	58.2% (53.0-63.3)	95.0% (86.8, 100)	65.7% (60.1-71.2)	96.7% (88.4-100)
Weighted & direct age adjustment	63.0%	95.0%	68.9%	97.3%
Weighted, direct age & sex adjustment*	64.2% (59.3-68.8)	95.5 (93.6, 97.5)	72.2% (67.6-76.3)	95.9% (92.2, 99.6)
Weighted excluding HIV infected individuals	63.7% (58.7-68.5)	-	70.1% (65.2-74.6%)	-

Notes: Point estimates and 95% logit confidence intervals represent IgG seroprevalence in the pre and post-campaign serosurvey. Equivocal results classified as positive. Estimates weighted based on survey design. Direct adjustments made to 2016 Southern Province population estimates from the Central Statistical Office. (*) indicates final seroprevalence estimates used as provincial level pre and post seroprevalence for measles and rubella.

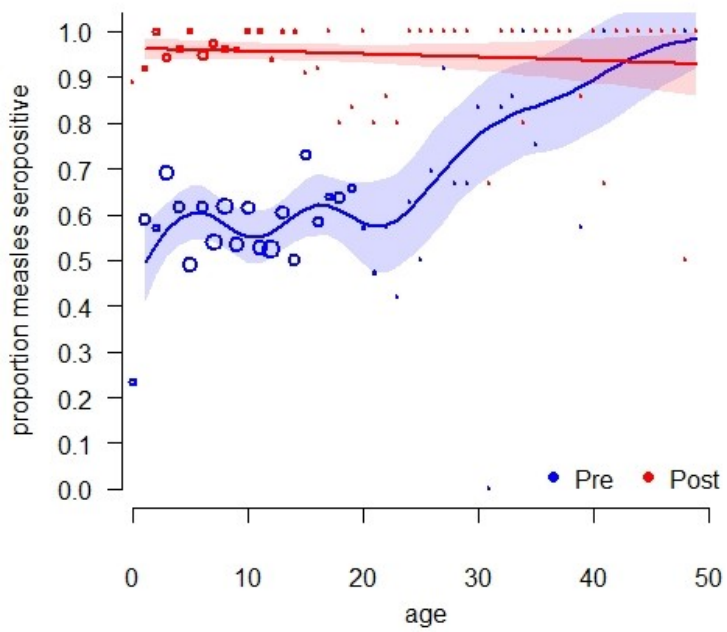
Figure 13. Model of factors associated with being included in the serological surveys, which affect seroprevalence estimates



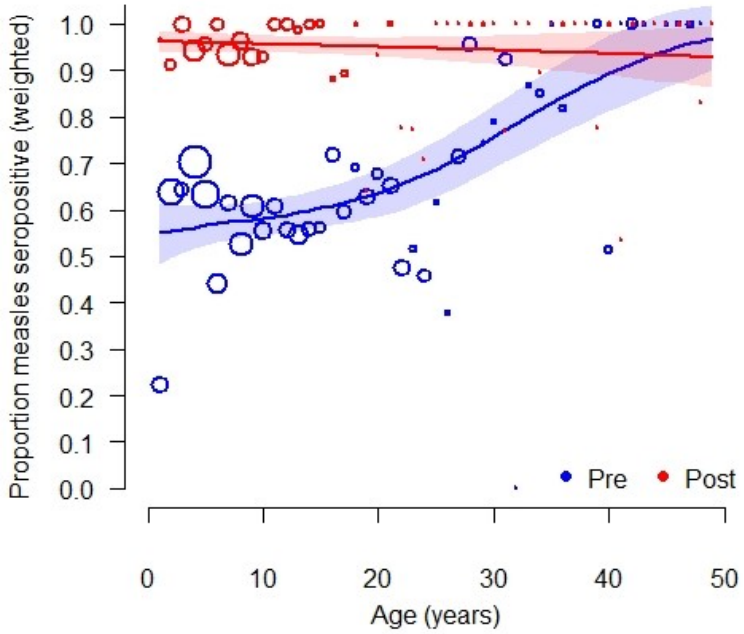
Notes: The primary criteria for inclusion in both the pre and post-campaign serosurveys was age. HIV status was part of the selection criteria for the pre-campaign serosurvey, and vaccination status was part of selection criteria for inclusion in the post-campaign serosurvey. Although sex was not explicitly part of selection criteria, there were differences by sex in who was eventually included in the serosurvey. Who is captured in the serosurvey directly affects the seroprevalence estimate generated by the serosurvey. Age and sex were collected in both serosurveys, so they were adjusted for in the overall seroprevalence estimates. Because HIV and vaccination status were not captured in both serosurveys, they could not be adjusted for in the seroprevalence estimates but were taken into account in the selection probability weighting.

Figure 14. Relationship of seropositivity and age in the pre and post-campaign serosurveys, polynomial regression spline

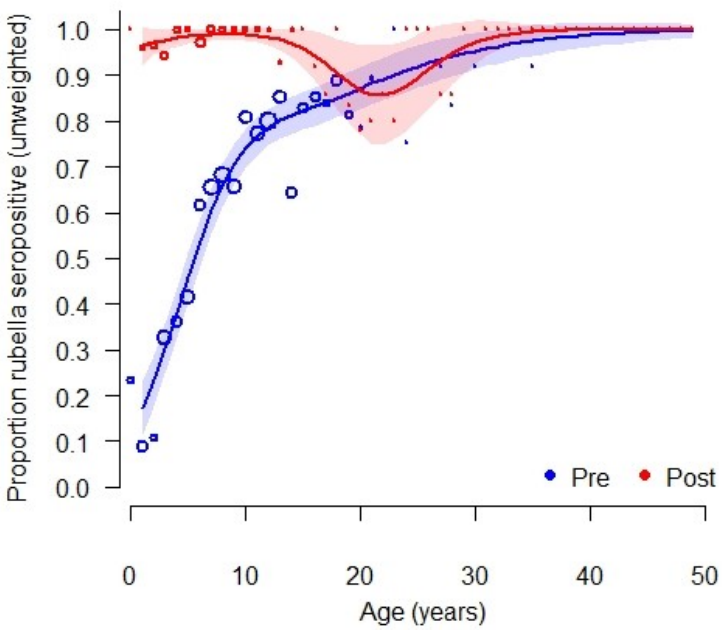
Measles seroprevalence by age (unweighted)



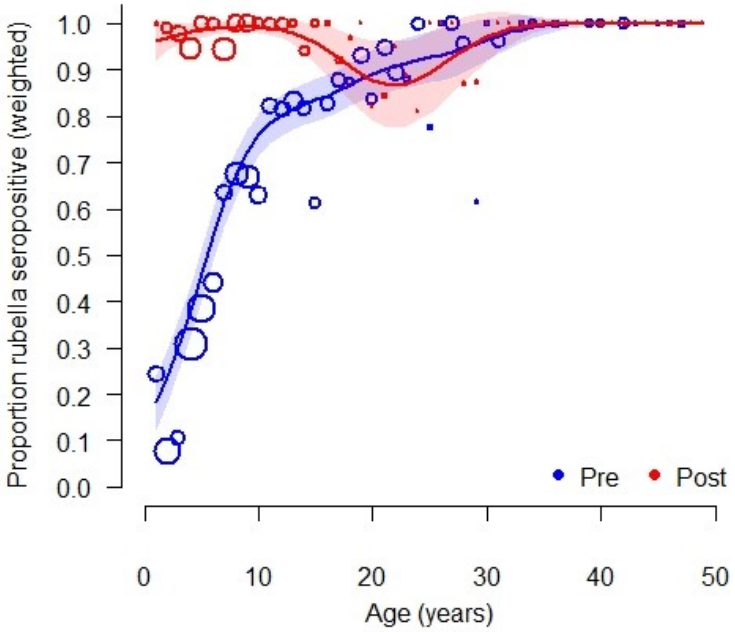
Measles seroprevalence by age (weighted)



Rubella seroprevalence by age (unweighted)

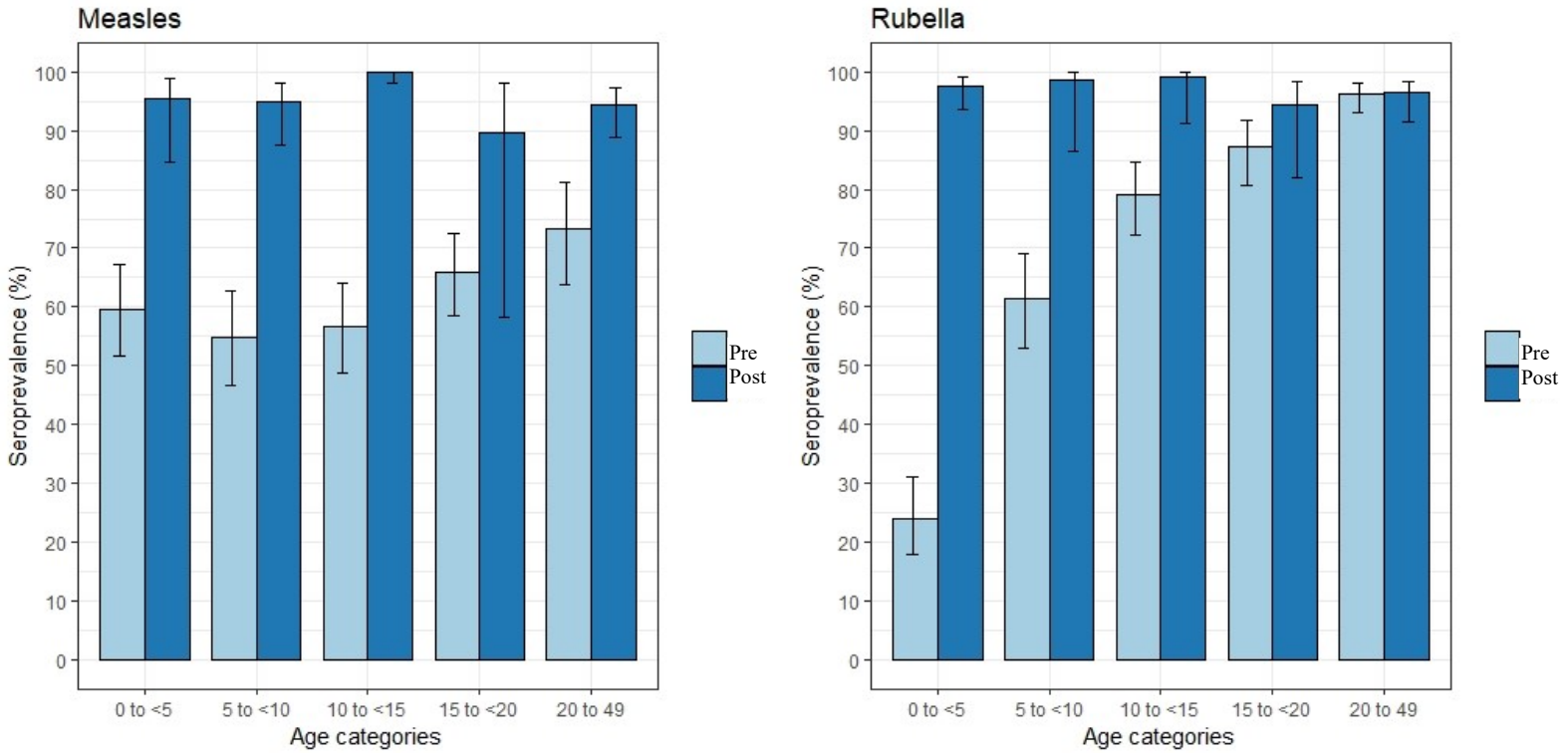


Rubella seroprevalence by age (weighted)



Notes: Blue and red lines represent the seroprevalence estimates for the pre and post-campaign serosurveys respectively. Size of circles are representative of sample size at each one-year age point. The lines represent the penalized regression spline fit, and shading represents 95% confidence intervals around the fit lines. Equivocal results were classified as seropositve. Weighting was done based on survey design

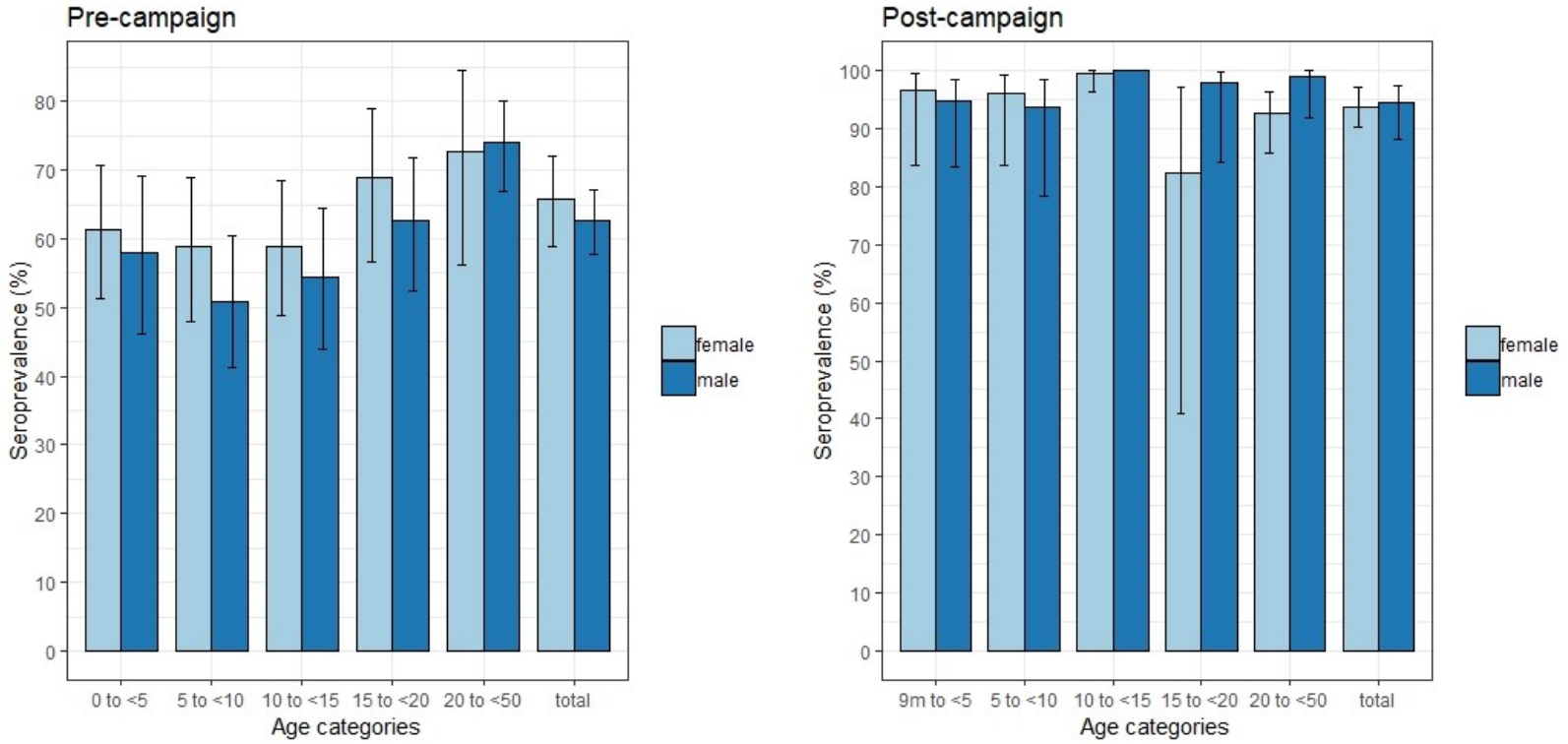
Figure 15. Seroprevalence estimates to measles and rubella by age categories pre and post measles-rubella vaccination campaign—weighted



Notes: Light and dark blue bars and 95% confidence intervals represent IgG seroprevalence in the pre and post-campaign serosurvey, respectively. Equivocal results classified as positive. Estimates weighted based on survey design. Age categories are in years.

Figure 16. Seroprevalence estimates to measles by age categories and sex pre and post measles-rubella vaccination campaign—weighted

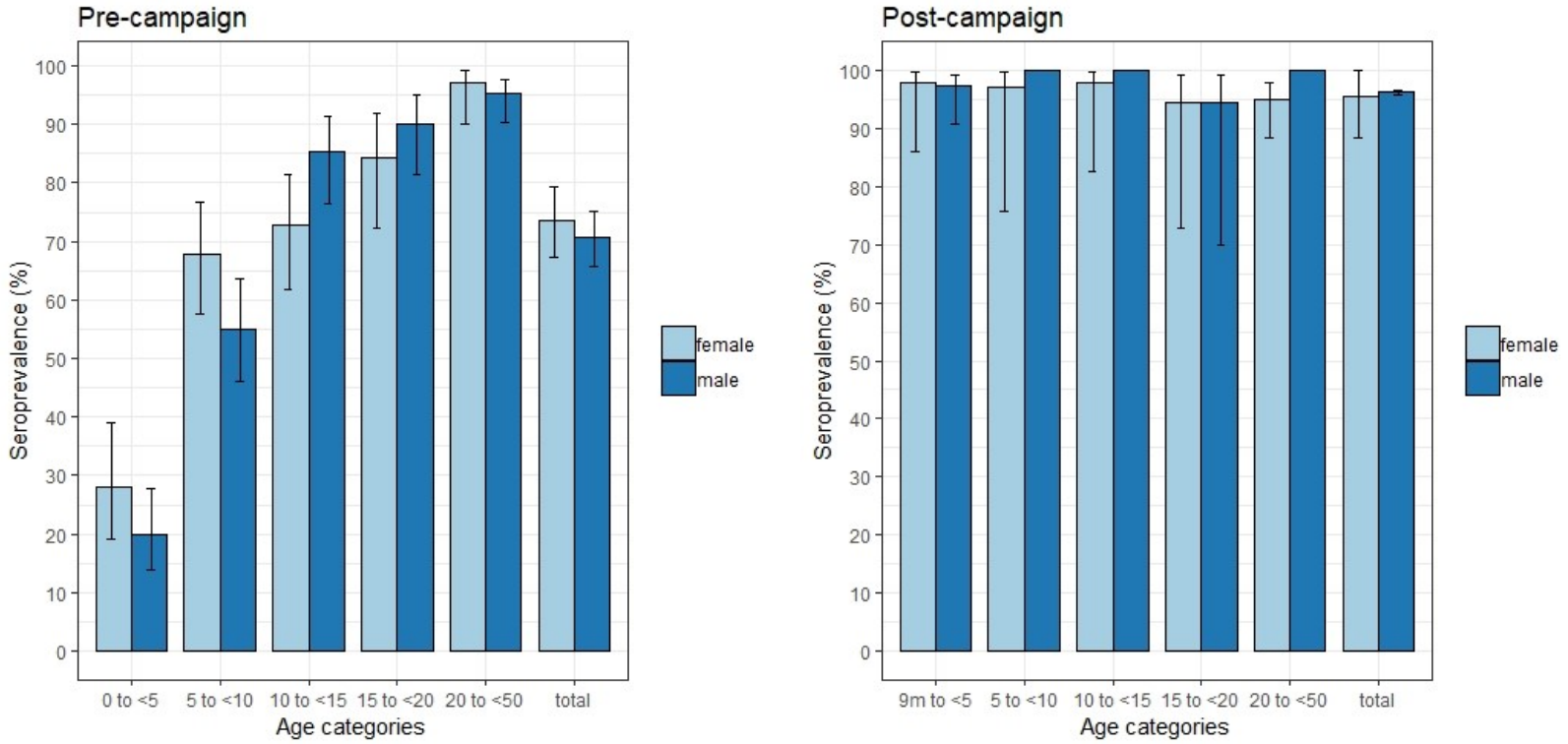
Measles



Notes: Light and dark blue bars and 95% confidence intervals represent IgG seroprevalence among females and males, respectively within each age category. 95% confidence intervals were not calculated for strata with 100% seroprevalence. Equivocal results classified as positive. Estimates were weighted based on survey design. Total is based on weighted and direct adjustment (for age and sex) to Southern Province population distribution in 2016. Age categories are in years unless otherwise noted.

Figure 17. Seroprevalence estimates to rubella by age categories and sex pre and post measles-rubella vaccination campaign—weighted

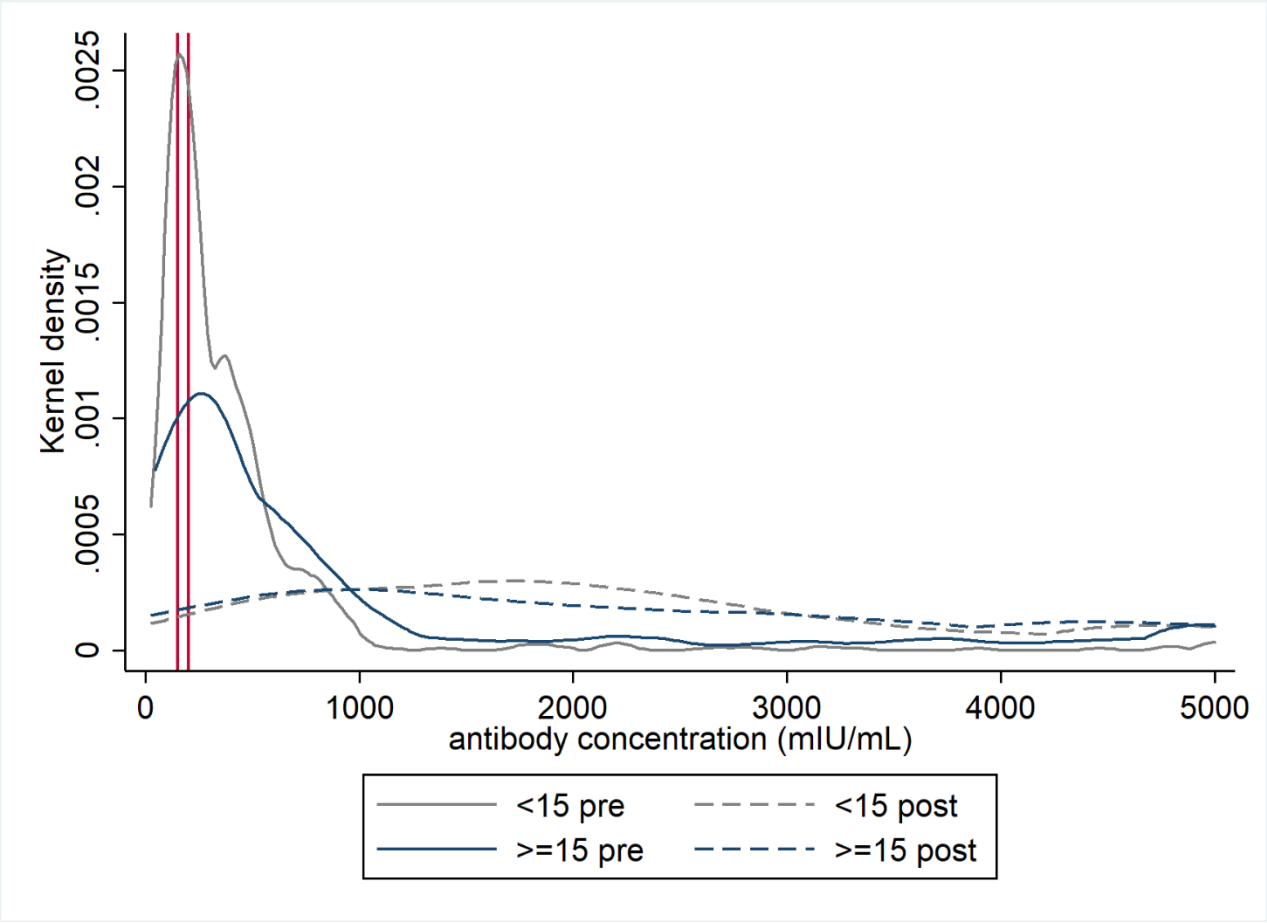
Rubella



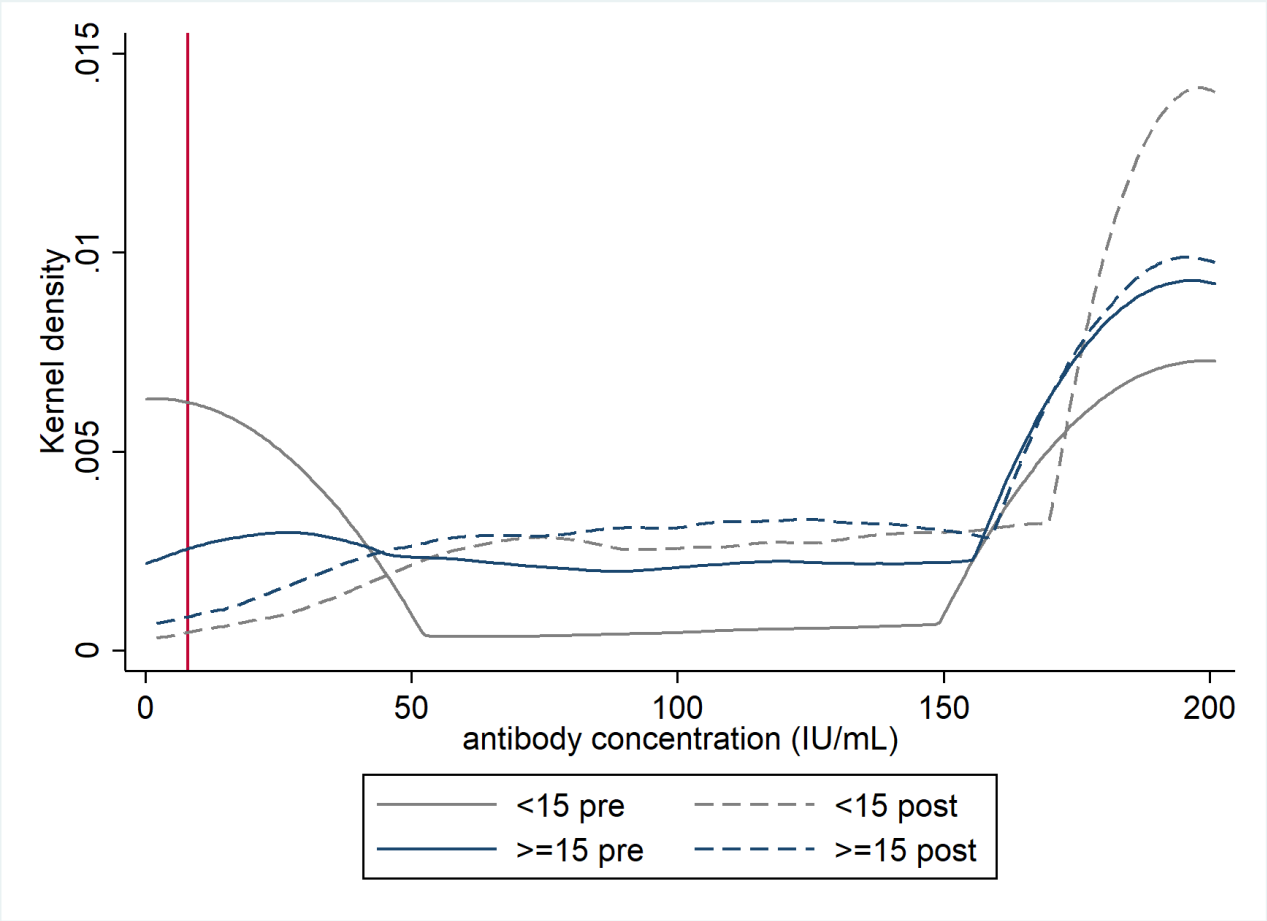
Notes: Light and dark blue bars and 95% confidence intervals represent IgG seroprevalence among females and males, respectively within each age category. 95% confidence intervals were not calculated for strata with 100% seroprevalence. Equivocal results classified as positive. Estimates were weighted based on survey design. Total is based on weighted and direct adjustment (for age and sex) to Southern Province population distribution in 2016. Age categories are in years unless otherwise noted.

Figure 18. Kernel density plot of IgG antibody concentrations pre and post measles-rubella vaccination campaign for participants by under 15-year olds and 15 years of age and older

Measles

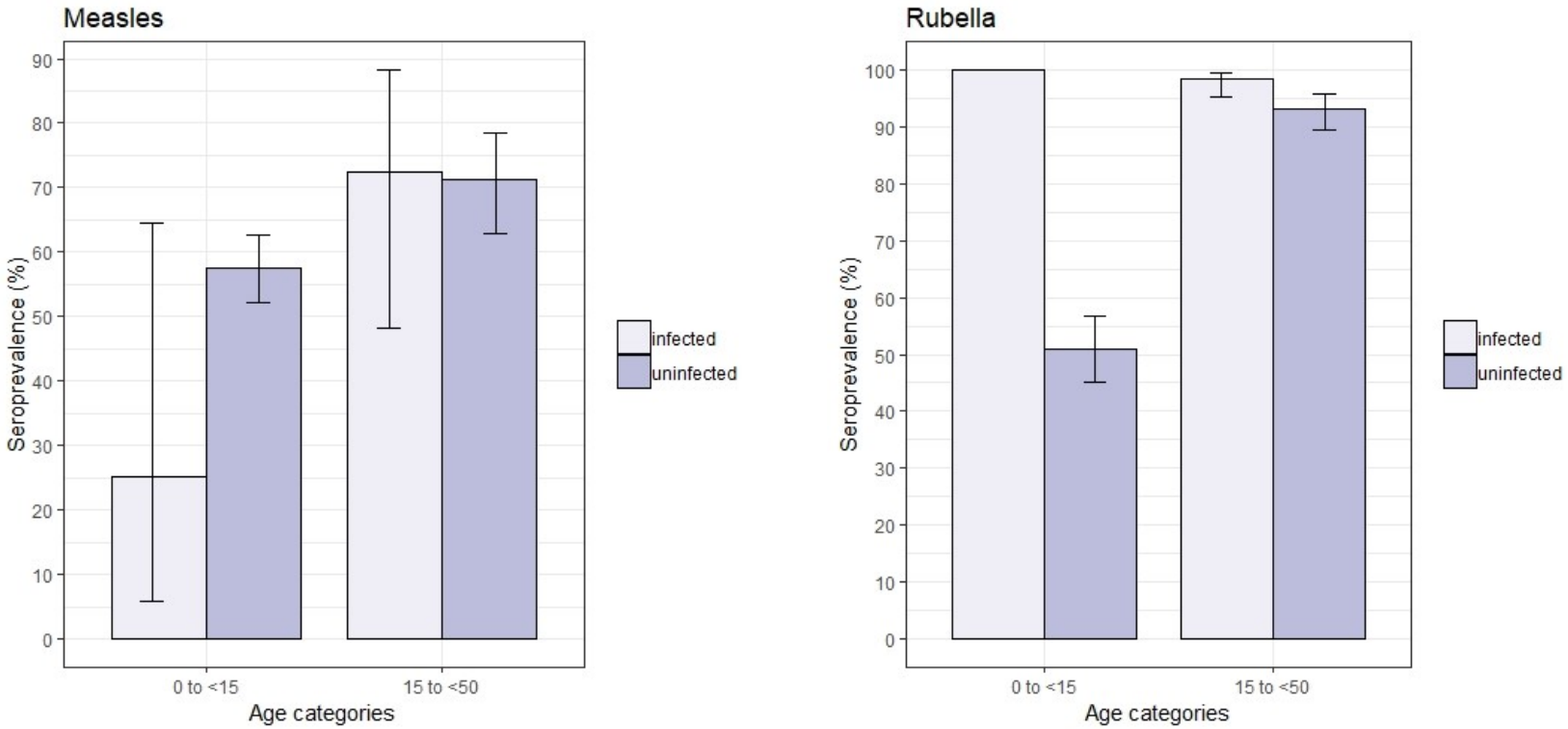


Rubella



Notes: Gray and navy colored lines represent the kernel density distribution of IgG antibody concentrations for participants under 15 years of age and 15 years of age and older respectively. The pre-campaign concentrations are represented by the solid lines, and post-campaign concentrations by dashed lines. Values are left-censored based on the lower limit of detection and right-censored based on the upper limit of detection. Kernel densities are the distribution of the samples in the corresponding serosurvey with that concentration. Red vertical line indicates upper cut-off value for assigning negatives (200 mIU/mL for measles pre serosurvey and 150 mIU/mL for measles post serosurvey, and 8 IU/mL for rubella). Equivocal results were analyzed as positives for both serosurveys.

Figure 19. Measles and rubella seroprevalence estimates among HIV infected and uninfected participants by under 15-year olds and 15 years of age and older



Notes: Light and dark purple bars and 95% confidence intervals represent IgG seroprevalence among HIV uninfected and infected, respectively within each age category. Equivocal results classified as positive. Estimates were weighted based on survey design. There were 9 HIV infected children under 15 years of age, and 734 uninfected. There were 34 HIV infected adults 15 years of age and older, and 328 uninfected. Age categories are in years unless otherwise noted.

Discussion

Public Health Importance

As countries strive for measles and rubella elimination, it may become more important to monitor population immunity to ensure high enough levels such that the introduction of virus would not result in ongoing transmission. While vaccination coverage has traditionally been used as a proxy, serological surveys provide a more accurate estimate of population immunity. Concordance between vaccination history and seropositivity has been found to be as low as 64.3% for measles (Polonsky et al., 2015). Serosurveys are also used by countries, such as China, Mongolia, New Zealand, Singapore and Japan, as evidence to present to regional elimination committees to monitor progress towards eliminating the transmission of measles or rubella (World Health Organization Regional Office for the Wesetern Pacific, 2017).

When choosing to conduct serological surveys, there are operational questions to be answered in terms of implementation. The better that serosurvey operational issues are understood and addressed, the more valid population immunity estimates can be derived. Sample size, selection criteria, representativeness, and testing methods all factor into the interpretability of seroprevalence estimates (World Health Organization Regional Office for the Wesetern Pacific, 2017). WHO released measles-rubella serosurvey guidelines that cover the process involved in planning, implementing, and analyzing data for serosurveys. While these guidelines provide the steps to conducting a serosurvey, they do not address concerns that serosurveys are costly and logistically challenging, making them difficult for low and middle-income countries

to implement. This study provides information to help guide programs that are planning serological surveys for measles and rubella.

One of the concerns with serosurveys is that there may be high refusal rates to biospecimen collection. We found that serosurveys were generally acceptable to the community in our setting. Additionally, there was a familiarity with the use of blood for health research, likely because serological specimens are collected for HIV and malaria program monitoring in this area. We also found that the preferred method of specimen collection in this area was finger prick blood collection. Using fingerpick blood collection in our serosurvey nested within a vaccination coverage survey may have helped prevent higher refusal rates in areas where biospecimens were collected, as compared to areas where they were not collected. Serosurveys in Zambia and similar settings should consider the use of fingerpick to make blood collection acceptable to the community.

Previous research on survey participation has used the health belief model to explain motivators for participation, which are primarily based on individual level perceptions. However, our findings show that the decision to participate in a serosurvey is not solely based on the individual being asked to participate, but rather an interplay of individual, interpersonal, and structural level factors, as depicted by the socioecological model. Providing information about the serosurvey ahead of time can alleviate concerns with serosurvey participation, but it needs to be done through a recognizable and trusted vehicle, such as the community health worker.

A unique consideration for many measles and rubella serosurveys is that they provide seroprevalence estimates at a community-level rather than individual-level serological status. However, we found that receiving test results was important to participants. Because point of care tests are not currently widely available, notifying an individual of their test results would be

resource-intensive and require reaching back out to participants after laboratory testing is completed. For this reason, we proposed providing community-level feedback, and most participants found this approach amenable. Future serosurveys could consider providing community feedback through community-wide meetings or notifying community leaders or community health workers of the aggregate results to may make serosurveys more acceptable to potential participants. Researchers would need to be careful providing feedback, communicating clearly that being seropositive to measles or rubella does not indicate the presence of disease, but rather protection. This differs from test results provided for HIV and malaria serosurveys, which provide disease status and refer for treatment. By contrast, notifying an individual that they are seronegative, could have ethical implications. If the individual is in an age group where they would be eligible for routine vaccination, they could be referred to the health post. However, participants outside the age range who are not protected against measles or rubella virus would have to seek vaccination in a private clinic, which could be expensive. Researchers would have to consider whether there is an ethical obligation to vaccinate participants who are notified as being seronegative and desire vaccination.

While it is often cited that serosurveys are costly to implement, there is limited empirical data on the actual cost of conducting serosurveys. Our estimate was much lower than the WHO estimate of $100,000-$1 million required for serosurveys. We also found that by nesting a serosurvey in a vaccination coverage survey, approximately two-thirds of the serosurvey cost can be saved. Given that current funding for measles-rubella vaccine introduction requires a post-campaign vaccination coverage survey, these coverage surveys provide an opportunity for specimen collection. Typically, there is a defined budget allocated for the post-campaign coverage survey, so programs would need to provide the additional funding for the inclusion of

serological specimen collection. If a program has allocated a certain amount for conducting a serosurvey, our findings can inform the sampling design based on the proposed budget. For example, the level of precision, number of clusters, and number of participants per cluster could be calculated to minimize the budget required. In a resource-constrained environment, such as in low-income countries, competing health priorities are an issue. If immunization programs can use findings from this study to design an efficient serosurvey, it could help advocate for the use of serosurveys.

To minimize cost and improve feasibility, we conducted our serosurveys as subsamples of larger surveys: (1) a subset of an HIV serosurvey biorepository and (2) a subset of participants in a vaccination coverage survey. While subsampling can make data collection more manageable and decrease time required for implementation, it can also make data analysis more challenging. Survey design weighting can account for the differential probability of selection of the subsamples as compared to the full survey samples. Secondary weighting of the full survey samples allows inferences to the target population. However, we found that in comparing results from serosurveys with different study designs and sampling methods, there were remaining differences. In order to compare the pre and post-campaign serosurveys, we needed to ensure seroprevalence estimates represented the same target population. We achieved this through direct age adjustment to the population estimated by then census. Because seroprevalence is significantly associated with age, serosurveys seeking to compare seroprevalence over time need to ensure a similar age distribution in order to be comparable. While the analysis may require additional weighting and adjustment, two serosurveys can be used to evaluate the effectiveness of a measles-rubella vaccination campaign.

The lower than expected seroprevalence to measles found in the pre-campaign serosurvey highlights another challenge with measles serosurveys. Similar to our findings, Kenya found that children under 15 years of age had only 60% seroprevalence to measles, which rose to 87% after a vaccination campaign (Ohuma et al., 2009). The cut-off value set by the commercial ELISA kit can have a major impact on the seroprevalence estimate, as demonstrated by the high proportion of specimens that fell close to the cut-off value. A European study found that the Euroimmun kit used during our pre-campaign serosurvey may underestimate positive measles results by 10%, thus underestimating seroprevalence to measles (Tischer et al., 2007).

We had the unique opportunity to conduct serosurveys prior to and just after the introduction of rubella containing vaccine. This allowed us to compare seroprevalence estimates from natural infection and vaccine-derived after the campaign in children who were eligible to have received vaccination. Interpreting serological results for measles and rubella can be more challenging after the introduction of vaccination. Comparing the patterns in the change to measles and rubella seroprevalence among children eligible for the campaign and adults who would not have been vaccinated helped shed light on some of the discrepancies between the two serosurveys. If a country conducts only one cross-sectional serosurvey, patterns of seroprevalence could not be ascertained.

The implications from these findings could also be generalizable to serological surveys conducted for other vaccine preventable diseases or infectious agents. For example, reasons associated with participation in a serological study may be applicable to other serosurveys. When planning for specimen collection in routine surveys such as the DHS or MICS, using DBS and making provisions for feedback of testing results could improve acceptability. The community's concern with who gets invited to participate can be addressed through communication messages

for a planned survey. Similarly, understanding the cost associated with increasing survey sample size to get more precise estimates by adding additional clusters or additional participants within clusters can also be translated to other cluster surveys. Finally, analytic challenges and design considerations to compare seroprevalence results from serosurveys using different study designs and sampling methods can also be used for serosurveys beyond measles and rubella. Adjusting for differential age or sex distributions can help make surveys comparable. These findings can help health programs better design serosurveys being planned in the future and how to best use serosurveys to monitor population immunity.

We collaborated with the Ministry of Health to ensure local perspective and priorities were being met in conjunction with our research goals and provide capacity building for serological surveillance. Furthermore, we used Zambian nationals to conduct all data collection and laboratory testing activities, demonstrating the capacity to perform laboratory testing in-country rather than shipping to reference laboratories. These findings provide a better characterization of the operational and feasibility issues of serological surveys to help decision makers design and implement serological surveys to meet their programmatic goals. Rather than being viewed as costly, logistically challenging endeavors, serosurveys can be more accessible for low and middle-income countries.

Limitations

Some of the issues with serological surveillance that this research does not directly address is the variability in laboratory assays. While we acknowledge the differences in the two ELISA kits used for the pre and post-campaign serosurvey, we did not conduct a direct head to head comparison of the assays. The potential difference between kits could imply that measles

seroprevalence pre-campaign was higher than our estimates; however, there has not been a direct comparison done between these two kits.

Furthermore, the serosurveys used in this analysis provide seroprevalence estimates, suggestive of population immunity. However, this is based on predetermined cutoffs defined by the ELISA kits, rather than true correlates of protection that could be obtained from plaque reduction neutralization tests (PRNT). However, it is not currently possible to conduct PRNT in Zambia. The use of commercially available ELISA kits makes serosurveys easier to implement in low- and middle-income countries, as not all may be able to perform PRNT.

The lower seroprevalence of measles among HIV infected children could also have affected our seroprevalence comparisons. Other high-HIV settings should consider whether testing for HIV should be incorporated into measles and rubella serosurveys, so it can be accounted for in the seroprevalence estimates.

In addition, current laboratory testing technology cannot distinguish whether an individual is seropositive to measles and rubella due to vaccination or natural infection. Seroprevalence estimates can provide a snapshot of population immunity at a single point in time. However, the proportion of seroprevalence due to vaccination programs cannot be distinguished from that due to circulating virus. Mathematical modeling may be help tease out the likelihood that immunity is vaccine versus infection induced. Repeated serosurveys, such as before and after a vaccination campaign, can also help provide information on the proportion of seroprevalence attributable to vaccination.

Finally, the generalizability of these findings in terms of serosurvey design, including acceptability considerations and cost, could be limited, as these results represent only one country in sub-Saharan Africa. Countries that are not used to household surveys or have higher

refusal rates to surveys could be more challenging to implement serosurveys. Similarly, countries where there may be more mistrust of government, research agencies, or Western medicine could also present challenges to serosurvey participation. Furthermore, Zambia is a setting with high HIV and malaria transmission, which is accustomed to blood collection for diagnostic purposes, so inferences may vary in other settings. As demonstrated in Paper 1, one-third of caregivers in this analysis had participated in previous surveys with specimen collection for HIV/AIDS or malaria. The existing infrastructure and laboratory capacity to conduct serosurveys for other diseases could also mean our costs underestimate what would be required in other settings.

Next Steps and Recommendation for Future Research

Serological surveillance for measles and rubella can be used to answer multiple research questions, from estimating burden of disease to predicting the risk of outbreaks. Here we present serological surveillance as a tool to identify immunity gaps. These serosurveys created immunological profiles in Zambia that can help identify vaccination targets to achieve and sustain elimination. The pre-campaign serosurvey demonstrated there were immunity gaps in participants under 30 years of age in Southern Province, justifying the need for a mass vaccination campaign in 2016 (Paper 3). The post-campaign serosurvey suggests that this immunity gap was closed, as seroprevalence at the end of 2016 was above 95%. There have also been no laboratory-confirmed cases of measles or rubella from 2016 to date, further suggesting that seroprevalence is high in Southern Province. However continued outbreaks in other parts of the country mean that Southern Province remains at risk of introduction of measles and rubella virus. Therefore, they must maintain high measles and rubella routine vaccination coverage to prevent future outbreaks. While this analysis depicted age-specific immunity gaps, serological

surveillance can also identify geographic immunity gaps. Ongoing analyses will use the nationwide specimens from the ZamPHIA study to identify spatial immunity gaps and assess spatial heterogeneity of measles and rubella seroprevalence.

Given that these serosurveys were conducted in 2016, mathematical modeling will be used with additional data sources, including surveillance and vaccination coverage, to predict 2019 immunological profiles. These models can help answer questions, such as the level of vaccination coverage with measles-rubella vaccine needed to prevent an increase in CRS cases after rubella vaccine introduction in 2016. Mathematical models with serological surveillance can also better predict the accumulation of individuals susceptible to measles than vaccination coverage and surveillance data. We found vaccination coverage data overestimated population immunity in the pre-campaign serosurvey, which could have been a factor of underestimated target populations, or revaccination of the same individuals. Therefore, serological surveillance may provide a more robust data source to predict population immunity gaps.

This information can be used to better target vaccination efforts (Lessler et al., 2016). For example, if a country like Zambia were considering doing a targeted vaccination campaign rather than a non-selective blanket vaccination campaign, serosurveys can provide population immunity estimates. This could be used to provide information in areas where there is a lack of confidence in vaccination coverage data and weak surveillance. Alternatively, if a country is considering excluding areas with strong routine immunization and no evidence of circulating virus, a serosurvey could verify that population immunity is in fact sufficiently high to provide herd immunity. As countries move away from external funding to self-financing, prioritization and allocation of resources for activities like vaccination campaigns will become critical.

One question that remains for the use of serological surveillance is whether they are cost-effective in terms of the better immunological profiles being worth the added cost. There is variability in the cost of serosurveys based on the sampling parameters, as noted in Paper 2 where costs could increase $10,000-$30,000 to move from +/-10% to +/-5% precision. However, the value of improving precision and the information gained from serosurveys is not clearly defined. If serosurveys could be used as noted above, to identify areas that may not need vaccination campaigns, they could be costed against the cost of a blanket vaccination campaign. Alternatively, serosurveys that identify immunity gaps and trigger vaccination response could be costed against what it would cost if an outbreak were to occur.

Given resource-constrained environments and the high proportion of a serosurvey cost spent on collecting specimens (Paper 2), we will be evaluating the ability to use other sources of biospecimens to estimate population immunity. The ZamPHIA serosurvey was one example of this, which will be costed. Other potential biorepositories being considered include antenatal care specimens and the demographic health survey. These biorepositories could identify population immunity gaps or provide trends over time, which could signal if population immunity declines.

We are also working to develop training modules to provide guidance if a country is interested in conducting a serosurvey. The costing framework (Paper 2) forms the basis to develop a budgeting tool for countries interested in serological surveillance. Lessons learned in terms of the acceptability of serological surveillance (Paper 1) will frame considerations for social mobilization efforts. Finally, the comparability of serosurveys (Paper 3) will feed into the serosurvey design and data analysis portions of the training, such as the need to collect a subset of serum or plasma if using dried blood spots, or post-stratification adjustments to improve

generalizability of results. These modules will provide capacity building to countries on how serological surveillance can guide immunization programs.

Conclusion

Serological surveillance can provide countries and program implementers with a more accurate population immunity estimate than vaccination coverage or fever rash surveillance. However, there are operational and methodological considerations that should be addressed when carrying out a serosurvey. Communities are willing to participate in serosurveys, and their decisions to participate are based on not only themselves, but also interpersonal factors and how the serosurvey is designed. Provisions to address concerns with serosurveys should include social mobilization through community health workers. The sampling design and methodology can decrease the cost of serosurveys, so they are not cost-prohibitive to low and middle-income countries. The difference in methodology between cross-sectional serosurveys can be accounted for through data analysis to demonstrate changes in seroprevalence. Immunity gaps identified through serological surveillance can then be used to target vaccination. The robust design and analysis of serosurveys provides valid population immunity estimates to help countries reach herd immunity thresholds to interrupt the transmission of measles and rubella viruses and achieve elimination.

Appendices

Appendix 1. Glossary of terms

Biomarker—a distinctive biological or biologically derived indicator of a process, event, or condition

Serosurvey – the collection and testing of specimens from a defined population over a specified period of time to estimate the prevalence of antibodies against a given etiologic agent as a direct measure of immunity

Seropositivity – detection in biological specimen of an antibody of a specific infectious disease or pathogen

Seroprevalence – the proportion of people in a population who test positive for antibodies against a specific infection or pathogen; it is often presented as a weighted percentage of the total specimens tested

Sero-protection - detection of antibody above a postulated protective threshold

Serosurveillance – serosurveys conducted routinely, periodically or through ongoing collection and testing of specimens to assess seroprevalence

*Definitions provided by (Cutts & Hanson, 2016)

Appendix 2. Interview and focus group guides from Paper 1

Interview Guide: Mixed-methods process evaluation of nested serological survey in Zambia
Interview guide version: v1.0 19Oct2016 (with research plan version)

Interview Subjects:

- Serosurvey supervisors
- Post-campaign coverage survey supervisors
- Southern Province post-campaign coverage survey coordinator
- Laboratory personnel
- Macha Research Trust personnel
- Serosurvey personnel

Context:

Mixed-methods process evaluation of the implementation of the serological survey

Specific language from protocol:

The analysis will focus on understanding the strengths and weaknesses of the nested serosurvey. Lessons learned in various aspects of implementation of the serosurvey will be examined, such as communication, collaboration with the PCES teams, best practices for collecting biospecimens, logistical issues and supervision needs.

Semi-structured Interview questions

Module I: Supervisors

Note to interviewer: The focus of these interviews is to understand the challenges and best practices of the individual during the execution of the nested serosurvey. We also want to identify how this integrated with the overall post-campaign coverage survey.

Core Questions:

1. Tell me about your experience as a supervisor in the nested serosurvey conducted during the post-campaign evaluation survey.

   Possible follow-up questions:
   - How did you first learn about/hear about the nested serosurvey in order to apply for the position?
   - How were you kept up to date about the progress of implementation? How frequently did you communicate with your team?

2. How were you involved in the preparations for the nested serosurvey?

   Possible follow-up questions:
   - How were you involved in the training for the nested serosurvey?
   - What could have been emphasized or modified in training to better prepare your team?

3. Could you tell me about your responsibilities during the serosurvey?

   Possible follow-up questions:
   - How clearly defined were your responsibilities at the beginning? Did they change over time? (*ask as two separate questions with time to answer the first one and then ask the second—not both together*)
   - How was coordination with the post-campaign coverage survey planning team before fieldwork?

4. How did the fieldwork for the serosurvey work for your team?

   Possible follow-up questions:
   - What did you think of the time needed to implement the serosurvey? What was your typical work schedule?
   - How was coordination with the post-campaign coverage survey teams?
     - Was it clear which households to enroll? (follow-ups: if not, why not? What was done to better clarify it during data collection? How could future preparations make this more clear?)
     - Was the serosurvey team able to keep up with the post-campaign coverage survey teams?

- How was the support provided to your team during fieldwork from the field coordinator and staff at Macha?
    - What role did the field coordinator and Macha staff play in helping to resolve issues?
    - What were some of the technical issues with the tablets, if any?
- How well were the teams able to perform blood specimen collection?
    - What were issues you noted in terms of conducting the fingerpick, dabbing blood spots?
    - What were issues you noted in terms of collecting liquid capillary blood tubes?
      (Probe for differences by age group and possible reasons why)
    - What were any concerns with the storage and transportation of specimens back to MRT?
    - Were there any adverse events that could have been foreseen?
- Were there any issues with the questionnaires? What were they?
    - Were there certain questions that were challenging to interpret?
    - How was data entry with the PCES team? Did they go too fast to capture the responses?
- Was there a clear delineation of roles and responsibilities between the PCES and your team?

5. How did the community perceive the nested serosurvey?

   Possible follow-up questions:
   - How did you communicate the serosurvey implementation plans ahead of time with the community?
   - How did the household react to receiving the teams in their homes for the survey?
   - What were people's questions when providing consent?
   - Were teams able to collect blood specimens from most household members? Were there certain members who tended to refuse more than others?
   - What effect has the nested serological survey had on your opinion of household surveys?

6. What lessons have you learned? We hope to implement future serosurveys; what would you do differently next time?

   Possible follow-up questions:
   - What were the strengths of the serosurvey? What went well? How were you able to achieve those things that went well?
   - What would have made the serosurvey more efficient?
   - Were any new operational/logistic strategies you put in place to improve efficiency during implementation?

Module II: Laboratory Personnel

1. Tell me about your experience as a supervisor in the nested serosurvey conducted during the post-campaign evaluation survey.

   Possible follow-up questions:
   - How were you kept up to date about the progress of implementation?
   - How frequently did you communicate with the field staff?

2. How was the condition of the DBS specimens?
   Note to Interviewer: Give informant plenty of time to address question before asking follow-up questions.

   Possible follow-up questions:
   - How were specimens transported to you? Please describe any challenges.
   - How timely were specimens received (i.e. within 24 hours, daily, at the end of a cluster)?
   - Where there differences in condition of different specimens?

3. How did you provide feedback to the fieldworkers?
   - What was the response of the teams?
   - Were you involved in the fieldwork? If so, how? What were your responsibilities? What would you usually have been doing during the time you spent on the serosurvey?

4. How was the condition of the liquid capillary blood specimens?

   Possible follow-up questions:
   - How were specimens transported to you? Please describe any challenges.
   - How timely were specimens received (i.e. within 24 hours)?
   - Was there any indication of cold chain issues?
   - Were there any opportunities for you to provide feedback to the fieldworkers? How did you provide feedback?

5. What were the challenges with specimen processing?

   Possible follow-up questions:
   - What were the differences between processing DBS and liquid capillary blood?
   - How were equivocal results treated?
   - What was the technical oversight or support provided?
   - What would have made it easier for you to perform your responsibilities?

6. What lessons have you learned from this serosurvey? Is there anything that could have been done differently?

   Possible follow-up questions:

- How could we have your role have been better suited to your other responsibilities?
- What things should supervisors and field workers do to ensure proper specimen collection? What things did the personnel do well? What could have been improved?

Guide for Discussion with Data Collectors

Instructions for Moderators (note that these instructions below and other specific instructions will be covered during trainings)

Note taking

- Take detailed notes to try to capture as much as possible of the discussion. Write down what each participant says, trying to capture exact quotes (do not write just summary statements from the discussion). **Use the recording device provided to ensure you capture the details.** The facilitator and note taker should write expanded notes immediately after the discussion (use the audio recordings). Recordings will be used to complement and be compared to the notes. The facilitator will also complement the expanded notes with a memo of his/her own observations or recollections
- Keep a note of "key words" mentioned by the respondents throughout the FGDs that could aid in the coding process.
- Final notes should be typed in a Word document and sent to Andrea Carcelen for analysis.

Using the discussion guide

- A list of questions is provided as a guide to lead the discussion (see below); however, they should not be asked as a questionnaire. The conversation should be allowed to flow naturally, and you should try to encourage everyone to voice their opinion. However, discussions on each question should not exceed 10 minutes. Whenever the conversation stops or needs to be redirected, you can do so by asking one of the questions that has not been discussed. Before the conversation ends, make sure that all the topics in the questions have been covered.

Time management

- Before the session, arrange for someone (not a participant) to act as time-keeper and make a plan for how you will be notified of the time.
- Manage the time so that each topic is covered, according to the time allowed.
- Use verbal cues to help keep time with statements like:
  - "There is only time for one more comment."
  - "This discussion is very important, but in the interest of time we need to end this part of the discussion now. We will have time for a final discussion later."

Agenda

Welcome, instructions and consent	5Mins
Ground rules and introductions	5Mins
Discussion	75 Mins
Close-out	5 Mins
Total time	90 Mins (1hr and 30 min)

Managing Group Dynamics

- The moderator's major goal is to collect useful information from all participants.
- Ensure even participation. If one or two people are dominating the discussion, then call on others to participate.
- Ensure only one person talks at a time and there is only one conversation occurring at a time (no side conversations).
- Promote mutual respect, especially when participants disagree. Remind them that everyone has a right to voice their point of view even if others do not agree.

Discussion Guide

Welcome, Instructions and Consent (5 minutes)

- **Introduction**
  Welcome and thank you for agreeing to participate in this focus group. My name is _____, and I'll be guiding today's discussion. I work with _____. To assist me with this activity are _____ (names of note takers and observers).

- **Purpose of Participation**
  As data collectors, you have insight into how the nested serosurvey was executed. We value your experience and want to listen to your views and hear your thoughts. The knowledge and the information we collect will help us to come up with improvements in implementing future serosurveys.

- **A comfortable participation for everyone:**
  We welcome all your comments, questions, and suggestions. We are eager to hear from each of you, but also want you to know that there is no obligation to answer any question that you do not feel comfortable answering. There is no right or wrong answer to the questions I'm going to ask, so please relax and feel free to speak openly.

- **Informed Consent and Confidentiality**
  Before we start, I would like everyone to understand that anything you say here will be kept anonymous and that there won't be any negative effects in terms of your contract or payment-based on what you say. This will not affect your payment or review in any way. Neither your name nor any information about you will be shared with any other person or organizations. No one outside of this group will ever know who said each comment; we will only share the summary of your combined responses and some anonymous statements. Please understand that there may be different opinions, but there should be mutual respect for these differences. I would also like to make sure everyone choosing to be a part of this focus group discussion willingly.

  (*Read the informed consent and answer any questions participants might have regarding their participation)*

(Ask if anyone wishes not to participate, and they will be excused to leave)

(Pause to see if anyone does not want to participate. If anyone does decide to leave, allow them to leave the room and then proceed.)

We would like to record our discussion today just to avoid my missing out on anything that you talk about during our conversation. My colleague will also take notes during the discussion. Does anyone object to that?

(Pause to see if anyone objects.)

Ground Rules and Introductions (5 minutes)

- **Ground Rules/Guidelines for Discussion:**

To make our discussion more comfortable and for it to run smoothly, there are a number of ground rules we will need to follow.

- Everyone's input is important.
- Please speak one at a time. Avoid side conversations.
- Please speak so that everyone can hear you. *[Remember, you are being audio taped. I may at times repeat what you say to make sure that your opinion is captured on tape.]*
- Stay focused on the question. *[I may need to cut a discussion short because of the limited time that we have. So please try to be brief.]*
- It is OK to disagree with another person's opinion or perspective. *[If you dislike something or disagree with something that is said, I want to hear about it. However, please avoid debating or trying to sway the opinion of others.*]
- Be respectful.

Introduction:

Is there anything else we need to clarify?

Thank you again for your willingness to participate. We know you have all put in a lot of hard work for this study, and want to ensure your perspective is heard.

Well, let's get started. OK, I'll start the recorder.

Discussion (50 minutes)

Overall

1. Could you tell me what it was like for you to participate in the nested serosurvey?

a. Probe: If you have participated in other surveys or studies, how did this compare to previous surveys you have participated in? What was the same/different?

Preparations

2. Please tell me what your impression of the training was
   a. Probe: "What could have been emphasized or modified to help you in the field?"
3. "Given that the PCES teams were charged with selecting the households, how clear were you on which households to enroll?"
   b. Probe: "Was this made clear to you in advance? How could it have been improved?"
   c. Probe: "What is your opinion about the coordination with the PCES teams? What do you think facilitated coordination with them?"

Now we are going to ask you some questions related to the actual implementation of the nested serosurvey.

4. How was the data collection process?
   d. How did the serosurvey affect the PCES activities? (*Probe timing of questionnaire, data recording and how well they were able to keep up with the PCES*)
   e. Were there any issues with the questionnaires?
   f. What type of supervision did you receive?
5. How was the blood specimen collection process?
   a. What were issues you noted in terms of conducting the fingerpick? What about dried blood spots? And if applicable to you, collecting liquid capillary blood tubes? (Pause between each question for a response)
   b. How was the storage and transportation of specimens back to MRT?
   c. Were there any adverse events you experienced?
      i. Probe: What strategies would you recommend for avoiding them in the future?
6. What do you think about how the community perceived the nested serosurvey?
   g. (Probe here for perceptions of "acceptability")
   h. (if there are concerns/issues, ask how should the concern(s) be addressed and probe for reasons of skepticism or hesitation and proposed solutions)
   i. If it were to be re-done, would you accept your child to participate in the serosurvey? Why or why not?
   (probe for "attitudes, risk perception, or structural concerns" that could explain the intention to participate)
7. What lessons have you learned as a surveyor in this study? We hope to implement serosurveys in other areas as well, so what would you do differently next time? (probe if not already discussed: training, implementation, specimen management, supervision
8. Is there anything else on these topics that you'd like to share with us today before we close?

Close-out **(5 minutes)**

Thank you again for your time today. Please recall that everything said here was confidential. This discussion has provided valuable information that will shape the future of serosurvey implementation.

Appendix 3. Post-introduction evaluation caregiver questionnaire for Paper 1

District:_____

Child's age: ___months
Maternal age:

- ☐ <30
- ☐ 30-39
- ☐ 40-49
- ☐ 50+

Religion:

- ☐ Catholic
- ☐ Pentecostal
- ☐ Protestant
- ☐ SDA
- ☐ Muslim
- ☐ Other

Time to vaccination clinic:

- ☐ <30 min
- ☐ 30-59 min
- ☐ >60min

1. Who influences your decisions about seeking healthcare? *[check all that apply]*
   - ☐ Family (spouse, parents, etc.)
   - ☐ Medical personnel (doctors, nurses)
   - ☐ Community health worker
   - ☐ Chief/headman/religious leaders
   - ☐ Other: _____

2. Have you received vaccine health information from community health workers (CHWs) in your area?
   - ☐ Yes
   - ☐ No
   - ☐ Don't know

   2a. What kind of information was provided?

   _____

   _____

3. Have you or a member of your family participated in any household health surveys (such as for vaccines, malaria, or HIV)?
   - ☐ Yes
   - ☐ No *[skip to question 4]*
   - ☐ Don't know *[skip to question 4]*

   3a. If yes: What the survey was for? *[check all that apply]*

- ☐ HIV
- ☐ Malaria
- ☐ Measles or rubella
- ☐ Other: _____

3b. During these surveys, did they collect any bodily fluids (such as blood)?

- ☐ Yes
- ☐ No *[skip to question 4]*
- ☐ Don't know *[skip to question 4]*

3c. If yes: Did you receive the test results?

- ☐ Yes
- ☐ No
- ☐ Don't know

3d. How often has your household been asked to participate in blood collection surveys?

- ☐ Never (0)
- ☐ Once (1)
- ☐ A few times (~2-4)
- ☐ Many times (>4)
- ☐ Don't know

4. If you were asked to participate in a study that collected a bodily fluid, which would you prefer?

> **Blood draw**= drawing a teaspoon of blood by putting a needle into a vein in your arm.
> **Mouth swab**= collecting saliva by rubbing a cotton swab along your gums for one and a half minutes.
> **Finger pricking**= collecting drops of blood by pricking your finger with a needle.

4a. Blood draw over finger pricking?

- ☐ Strongly prefer
- ☐ Prefer
- ☐ No preference
- ☐ Do NOT prefer
- ☐ Strongly do NOT prefer

4b. Finger pricking over mouth swab?

- ☐ Strongly prefer
- ☐ Prefer
- ☐ No preference
- ☐ Do NOT prefer
- ☐ Strongly do NOT prefer

4c. Mouth swab over blood draw?

- ☐ Strongly prefer
- ☐ Prefer
- ☐ No preference
- ☐ Do NOT prefer
- ☐ Strongly do NOT prefer

4d. Why do you prefer any one of these?

5. How likely are you to accept collecting finger prick blood from yourself at home?
    - ☐ Very likely
    - ☐ Somewhat likely
    - ☐ Unlikely
    - ☐ Not at all likely
6. How likely are you to accept having finger prick blood collected from your children at home?
    - ☐ Very likely
    - ☐ Somewhat likely
    - ☐ Unlikely
    - ☐ Not at all likely
7. How important is it to you to receive the test results for you or your child?
    - ☐ Very important
    - ☐ Somewhat important
    - ☐ Not important
    - ☐ Not at all important

    7a. Would it be acceptable to give summary results to the district health officer instead of providing you individual results? (for example, 90% of the people in this village are protected against measles)
    - ☐ Very acceptable
    - ☐ Acceptable
    - ☐ Not acceptable
    - ☐ Very not acceptable
8. In your opinion, what are some reasons people may refuse blood collection in their households? *[check all that apply]*
    - ☐ The amount of blood collected
    - ☐ Being unsure what is going to be done with the blood (why want the blood)
    - ☐ Not getting test results
    - ☐ Religious beliefs prevent blood collection
    - ☐ Fear of needles
    - ☐ Safety concerns (for example, that equipment is not clean)
    - ☐ Too many researchers collecting blood from community
    - ☐ Distrust of the government
    - ☐ Other: _____
9. In your opinion, what would make people more likely to allow finger prick in their households? *[check all that apply]*
    - ☐ Social mobilization through community health workers (CHWs)
    - ☐ Community members trust their CHW
    - ☐ Community desire for blood to be collected because interested in test results
    - ☐ Community thinks they will get some sort of benefit (medical treatment, money)
    - ☐ Community is used to having blood collected
    - ☐ Desire to know more about health issues
    - ☐ Desire to better society/community
    - ☐ Other: _____

Appendix 4. Post-campaign serosurvey data collection questionnaire

COMPLETE FOR CHILDREN AGES 9 MONTHS TO 4 YEARS AT TIME OF M-R VACCINATION CAMPAIGN *ONLY*

Annex 2D: Cluster Survey Form – ROUTINE EPI SURVEY

EPI CLUSTER SURVEY FORM - CHILD AGED **9 months to 4 years at time of M-R vaccination campaign** Cluster #: _____ Cluster name: _____ Household #: _____ Date (DD/MM/YY): _____							
BIRTH DATE RANGE **From:** 7th December 2011 **To:** 19th January 2016		**NAME OF CHILD**					
Participant ID number							
Birth Date of child: (DD/MM/YY)							
Evidence of immunisation card	Yes/No						
BCG0	Date/Yes/No						
Is scar seen?	Yes/No						
OPV0	Date/Yes/No						
OPV1	Date/Yes/No						
Penta1	Date/Yes/No						
PCV1	Date/Yes/No						
Rota1	Date/Yes/No						
OPV2	Date/Yes/No						
Penta2	Date/Yes/No						
Rota2	Date/Yes/No						
PCV2	Date/Yes/No						
OPV3	Date/Yes/No						
Penta3	Date/Yes/No						
PCV3	Date/Yes/No						
IPV	Date/Yes/No						
Measles First dose	Date/Yes/No						
Measles Second dose	Date/Yes/No						
Measles campaign dose	Date/Yes/No						

COMPLETE FOR ALL PEOPLE IN HOUSEHOLD AGES 9 MONTHS AND ABOVE AT TIME OF M-R VACCINATION CAMPAIGN

PART 2E/F: DEMOGRAPHIC, HEALTH HISTORY, BLOOD COLLECTION – ALL PARTICIPANTS

CLUSTER NUMBER: _____ **HOUSEHOLD NUMBER: _____** **TODAY'S DATE (DD/MM/YY): _____**

Demographic & health information									Blood collection				
Participant ID	Participant date of birth (DD/MM/YY) if only month and year (MM/YY) If only year (YYYY)	Participant age in months if less than age 1 yr	Participant age in years, if 1 year or older	Sex	If female, have you ever been pregnant?	How many times have you ever been pregnant?	If 1 or more pregnancies, did you go to a clinic or hospital for antenatal care (ANC)?	Have you ever gotten a vaccination to prevent tetanus, usually an injection in the arm?	Was blood collected from this partici-pant?	If not, why was blood not collected?	What kind of blood was collected? Check all appropriate boxes.	Outcome of liquid blood collection Note: sufficient = at least 250 uL	How many dried blood spots were filled?
				0. male 1. female	0. No 1. Yes		0. No, never 1. Yes for every pregnancy 2. Yes for some pregnancies	0. No 1. Yes 2. Don't know	0. No 1. Yes	1. Refusal before blood collection 2. Refusal during blood collection 3. Individual too sick to give a sample 4 . Reached maximum number of fingerpicks 5 . Other reason. Describe…	1. Liquid capillary blood 2. Dried blood spots (DBS) 3. Both liquid and DBS	1. Sufficient blood volume 2. Could not get enough blood volume	0-5

Appendix 5. Quality control procedures for pre-campaign serosurvey laboratory testing

To monitor laboratory testing procedures, approximately 8% of specimens were systematically retested on the same plate (7 specimens from each initial plate). In addition, a different 5% of specimens (4 specimens from each initial plate) were retested on different plates to assess reproducibility. Additionally, two internal control specimens were tested on every plate to monitor comparability between plates, in addition to the negative and positive controls included in the testing kits.

Figure 1. Measles repeat testing on the same plate (R^2=95.6%)

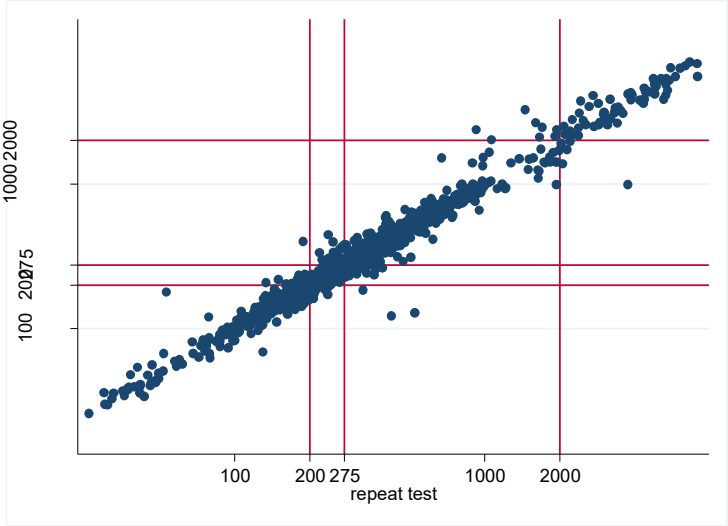
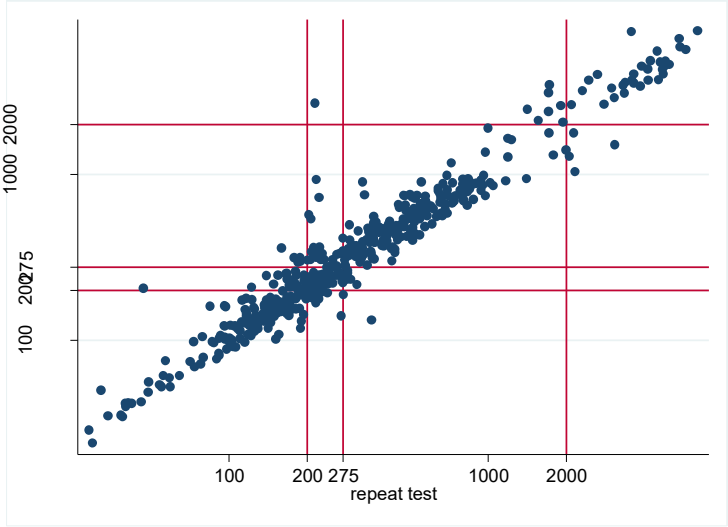


Figure 2. Measles repeat testing on different plates (R^2=91.8%)



Notes: Values are IgG antibody concentrations for measles (mIU/mL) during initial and repeated test for all specimens repeat tested. Each dot represents one specimen. Red lines designate upper cut-off values for negative (200mIU/mL) and equivocal (275 mIU/mL).

Figure 3. Rubella repeat testing on the same plate (R^2=95.7%)

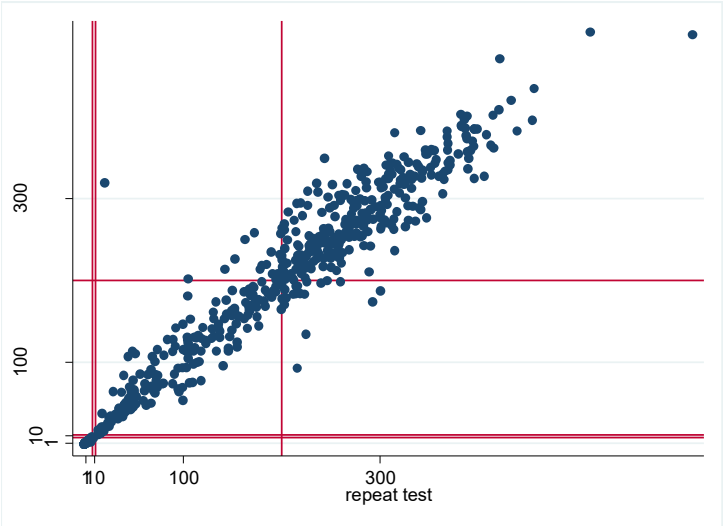
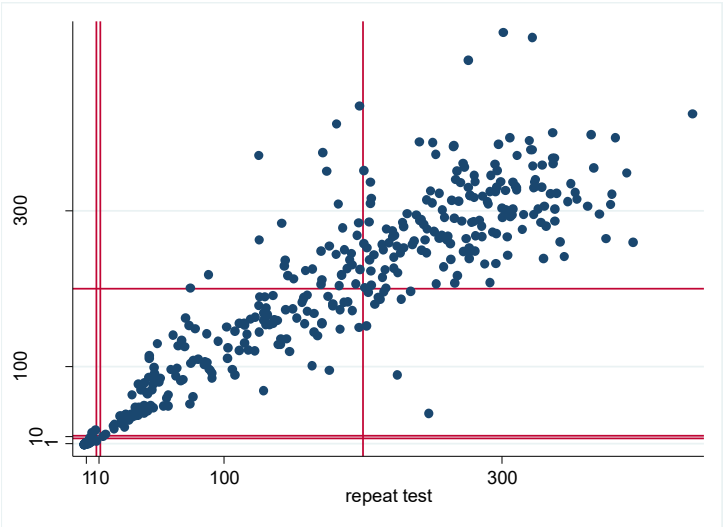


Figure 4. Rubella repeat testing on different plates (R^2=87.2%)



Notes: Values are IgG antibody concentrations for rubella (IU/mL) during initial and repeated test for all specimens repeat tested. Each dot represents one specimen. Red lines designate upper cut-off values for negative (8mIU/mL) and equivocal (10 IU/mL).

Appendix 6. Pre-campaign measles serosurvey laboratory testing with alternate cut-off value

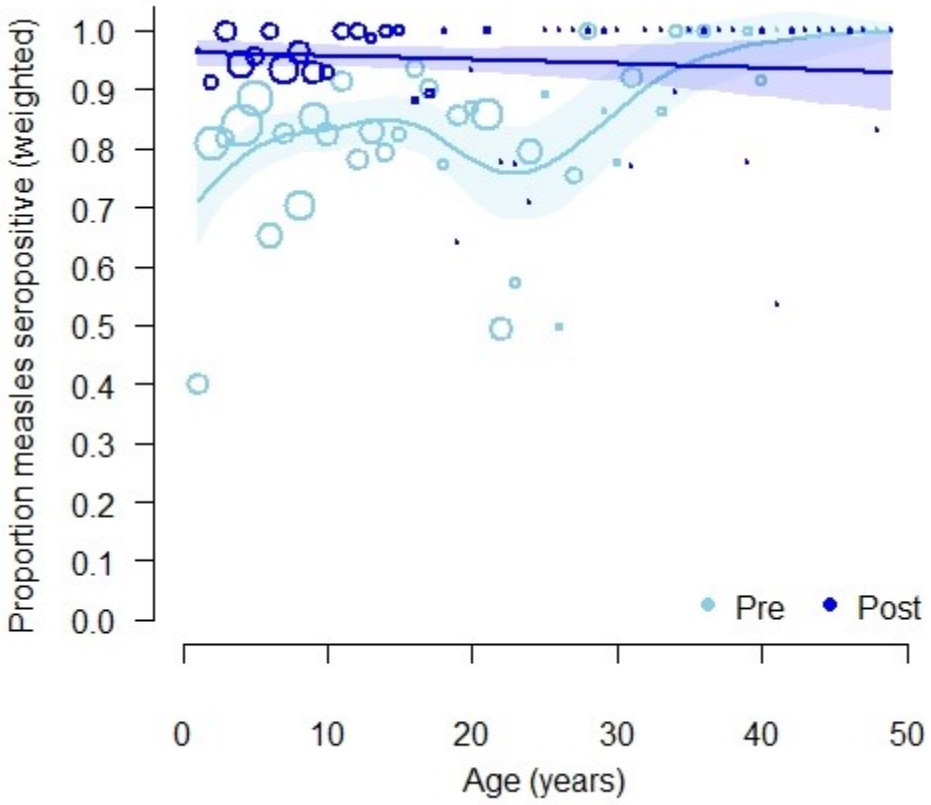
Given the lower sensitivity of the Euroimmun measles kit compared to other ELISA kits, we considered lowering the threshold for equivocals down to 150 mIU/mL. The results demonstrated that there was increase in overall measles seroprevalence in the pre-campaign frp, 64.2% to 82.0% with the new cut-off. By age-category, there is no longer a statistically significant difference in the pre and post campaign in the age categories older than 15 years of age, as would be expected since they were outside the range of the vaccination campaign.

Table 1. Seroprevalence to measles by age category in the pre and post-campaign serosurveys using 200 mIU/mL and 150 mIU/mL cut-offs in the pre-campaign serosurvey

Age category	Pre (200 mIU/mL)	Pre (150 mIU/mL)	Post
<5	59.7* (51.6-67.3)	77.9* (69.9-84.3)	95.5 (84.6-98.8)
5-9	54.8* (46.7-62.7)	77.0* (70.3-82.5)	95 (87.4-98.1)
10-14	56.6* (48.8-64.1)	83.0* (76.9-87.7)	99.8 (98.1-99.9)
15-19	65.8* (58.4-72.5)	87.5 (81.8-91.5)	89.6 (58.3-98.2)
20-49	73.3* (63.8-81.1)	84.6 (76.1-90.4)	94.3 (88.8-97.2)
Total	64.2* (59.3-68.8)	82.0 (78.1-85.4)	95.5 (93.6-97.5)

Note: Value indicates population seroprevalence estimate in that age category and 95% confidence interval. (*) indicates whether there was a statistically significant difference between the pre and post-campaign seroprevalence estimates.

Figure 1. Relationship of measles seropositivity and age in the pre and post-campaign serosurveys, polynomial regression spline



Notes: Light blue and dark blue lines represent the seroprevalence estimates for the pre and post-campaign serosurveys respectively. Size of circles are representative of sample size at each one-year age point. The lines represent the penalized regression spline fit, and shading represents 95% confidence intervals around the fit lines. Equivocal results were classified as seropositve. Weighting was done based on survey design.

REFERENCES

Almeida, L., Azevedo, B., Nunes, T., Vaz-da-Silva, M., & Soares-da-Silva, P. (2007). Why healthy subjects volunteer for phase I studies and how they perceive their participation? *Eur J Clin Pharmacol, 63*(11), 1085-1094. doi:10.1007/s00228-007-0368-3

Amina, M. D., Oladapo, S., Habib, S., Adebola, O., Bimbo, K., & Daniel, A. (2010). Prevalence of rubella IgG antibodies among pregnant women in Zaria, Nigeria. *Int Health, 2*(2), 156-159. doi:10.1016/j.inhe.2010.03.004

Babigumira, J. B., Morgan, I., & Levin, A. (2013). Health economics of rubella: a systematic review to assess the value of rubella vaccination. *BMC Public Health, 13*, 406. doi:10.1186/1471-2458-13-406

Barreto, J., Sacramento, I., Robertson, S. E., Langa, J., de Gourville, E., Wolfson, L., & Schoub, B. D. (2006). Antenatal rubella serosurvey in Maputo, Mozambique. *Trop Med Int Health, 11*(4), 559-564. doi:10.1111/j.1365-3156.2006.01577.x

Bland, J., & Clements, J. (1998). Protecting the world's children: the story of WHO's immunization programme. *World Health Forum, 19*(2), 162-173.

Bryant Borders, A. E., Grobman, W. A., Amsden, L. B., Collins, E. T., & Holl, J. L. (2007). Factors that influence the acceptability of collecting in-home finger stick blood samples in an urban, low-income population. *J Health Care Poor Underserved, 18*(1), 100-115. doi:10.1353/hpu.2007.0004

CDC. (2001). From the Centers for Disease Control and Prevention. Measles incidence before and after supplementary vaccination activities--Lusaka, Zambia, 1996-2000. *JAMA, 286*(4), 411-413.

CDC. (2005). Progress in measles control--Zambia, 1999-2004. *MMWR Morb Mortal Wkly Rep, 54*(23), 581-584.

Centers for Disease Control and Prevention. (2005). Progress in measles control--Zambia, 1999-2004. *MMWR Morb Mortal Wkly Rep, 54*(23), 581-584.

Charmaz, K. (2006). Coding in Grounded Theory Practice. In *Constructing Grounded Theory: A Practical Guide Through Qualitative Analysis* (pp. 42-71). London, UK: SAGE Publications Ltd.

Chatio, S., Baiden, F., Achana, F. S., Oduro, A., & Akazili, J. (2016). Knowledge and Perceptions about Clinical Trials and the Use of Biomedical Samples: Findings from a Qualitative Study in Rural Northern Ghana. *PLoS One, 11*(4), e0152854. doi:10.1371/journal.pone.0152854

Clarke, M., Schild, G. C., Boustred, J., McGregor, I. A., & Williams, K. (1980). Epidemiological studies of rubella virus in a tropical African community. *Bull World Health Organ, 58*(6), 931-935.

Colson, K. E., Potter, A., Conde-Glez, C., Hernandez, B., Rios-Zertuche, D., Zuniga-Brenes, P., . . . Mokdad, A. H. (2015). Use of a commercial ELISA for the detection of measles-specific immunoglobulin G (IgG) in dried blood spots collected from children living in low-resource settings. *J Med Virol, 87*(9), 1491-1499. doi:10.1002/jmv.24136

Colson, K. E., Zuniga-Brenes, P., Rios-Zertuche, D., Conde-Glez, C. J., Gagnier, M. C., Palmisano, E., . . . Mokdad, A. H. (2015). Comparative Estimates of Crude and Effective Coverage of Measles Immunization in Low-Resource Settings: Findings from Salud Mesoamerica 2015. *PLoS One, 10*(7), e0130697. doi:10.1371/journal.pone.0130697

Columbia University. (2016, December 2016). Zambia population-based HIV impact assessment: ZAMPHIA 2015-2016. Retrieved from http://phia.icap.columbia.edu/wp-content/uploads/2016/09/ZAMBIA-Factsheet.FIN_.pdf

Coppeta, L., Morucci, L., Pietroiusti, A., & Magrini, A. (2019). Cost-effectiveness of workplace vaccination against measles. *Hum Vaccin Immunother*, 1-4. doi:10.1080/21645515.2019.1616505

Cutts, F. T., & Hanson, M. (2016). Seroepidemiology: an underused tool for designing and monitoring vaccination programmes in low- and middle-income countries. *Trop Med Int Health, 21*(9), 1086-1098. doi:10.1111/tmi.12737

Cutts, F. T., Izurieta, H. S., & Rhoda, D. A. (2013). Measuring coverage in MNCH: design, implementation, and interpretation challenges associated with tracking vaccination coverage using household surveys. *PLoS Med, 10*(5), e1001404. doi:10.1371/journal.pmed.1001404

Cutts, F. T., & Vynnycky, E. (1999). Modelling the incidence of congenital rubella syndrome in developing countries. *Int J Epidemiol, 28*(6), 1176-1184.

Dabbagh, A., Laws, R. L., Steulet, C., Dumolard, L., Mulders, M. N., Kretsinger, K., . . . Goodson, J. L. (2018). Progress Toward Regional Measles Elimination - Worldwide, 2000-2017. *MMWR Morb Mortal Wkly Rep, 67*(47), 1323-1329. doi:10.15585/mmwr.mm6747a6

Dhalla, S., & Poole, G. (2014). Motivators to participation in actual HIV vaccine trials. *AIDS Behav, 18*(2), 263-277. doi:10.1007/s10461-013-0519-8

Dimech, W., & Mulders, M. N. (2016a). A 16-year review of seroprevalence studies on measles and rubella. *Vaccine, 34*(35), 4110-4118. doi:10.1016/j.vaccine.2016.06.002

Dimech, W., & Mulders, M. N. (2016b). A review of testing used in seroprevalence studies on measles and rubella. *Vaccine, 34*(35), 4119-4122. doi:10.1016/j.vaccine.2016.06.006

Duintjer Tebbens, R. J., Diop, O. M., Pallansch, M. A., Oberste, M. S., & Thompson, K. M. (2019). Characterising the costs of the Global Polio Laboratory Network: a survey-based analysis. *BMJ Open, 9*(1), e023290. doi:10.1136/bmjopen-2018-023290

Durrheim, D. N., Orenstein, W. A., & Schluter, W. W. (2018). Assessing population immunity for measles elimination - The promise and peril of serosurveys. *Vaccine, 36*(28), 4001-4003. doi:10.1016/j.vaccine.2018.04.036

Edmunds, W. J., Gay, N. J., Kretzschmar, M., Pebody, R. G., Wachmann, H., & Network, E. P. E. S.-e. (2000). The pre-vaccination epidemiology of measles, mumps and rubella in Europe: implications for modelling studies. *Epidemiol Infect, 125*(3), 635-650. doi:10.1017/s0950268800004672

Erondu, N. A., Ferland, L., Haile, B. H., & Abimbola, T. (2019). A systematic review of vaccine preventable disease surveillance cost studies. *Vaccine, 37*(17), 2311-2321. doi:10.1016/j.vaccine.2019.02.026

Ferson, M. J., Robertson, P. W., & Whybin, L. R. (1994). Cost effectiveness of prevaccination screening of health care workers for immunity to measles, rubella and mumps. *Med J Aust, 160*(8), 478-482.

Gastanaduy, P. A. R., Susan B.; Clemmons, Nakia S.; Lee, Adria D.; Hickman, Carol J.; Rota, Paul; Patel, Manisha. (2013). Chapter 7: Measles. In CDC (Ed.), *Manual for the Surveillance of Vaccine-Preventable Diseases* (6 ed.). Atlanta, GA: CDC.

GAVI. (2018). Zambia. Retrieved from http://www.gavi.org/country/zambia/#

Glaser, B. G. S. A. L. (1967). *The Discovery of Grounded Theory*. New Brunswick, USA: Aldine Transaction.
Gomwalk, N. E., & Ahmad, A. A. (1989). Prevalence of rubella antibodies on the African continent. *Rev Infect Dis, 11*(1), 116-121. doi:10.1093/clinids/11.1.116
Goodson, J. L., Masresha, B., Dosseh, A., Byabamazima, C., Nshimirimana, D., Cochi, S., & Reef, S. (2011). Rubella epidemiology in Africa in the prevaccine era, 2002-2009. *J Infect Dis, 204 Suppl 1*, S215-225. doi:10.1093/infdis/jir108
Guerra, F. M., Bolotin, S., Lim, G., Heffernan, J., Deeks, S. L., Li, Y., & Crowcroft, N. S. (2017). The basic reproduction number (R0) of measles: a systematic review. *Lancet Infect Dis, 17*(12), e420-e428. doi:10.1016/S1473-3099(17)30307-9
Hardelid, P., Williams, D., Dezateux, C., Cubitt, W. D., Peckham, C. S., Tookey, P. A., & Cortina-Borja, M. (2008). Agreement of rubella IgG antibody measured in serum and dried blood spots using two commercial enzyme-linked immunosorbent assays. *J Med Virol, 80*(2), 360-364. doi:10.1002/jmv.21077
Harvey, S. A., Jennings, L., Chinyama, M., Masaninga, F., Mulholland, K., & Bell, D. R. (2008). Improving community health worker use of malaria rapid diagnostic tests in Zambia: package instructions, job aid and job aid-plus-training. *Malar J, 7*, 160. doi:10.1186/1475-2875-7-160
Hayford, K., Mutembo, S., Carcelen, A., Matakala, H. K., Munachoonga, P., Winter, A., . . . Moss, W. J. (2019). Measles and rubella serosurvey identifies rubella immunity gap in young adults of childbearing age in Zambia: The added value of nesting a serological survey within a post-campaign coverage evaluation survey. *Vaccine, 37*(17), 2387-2393. doi:10.1016/j.vaccine.2019.02.037
Helfand, R. F., Keyserling, H. L., Williams, I., Murray, A., Mei, J., Moscatiello, C., . . . Bellini, W. J. (2001). Comparative detection of measles and rubella IgM and IgG derived from filter paper blood and serum samples. *J Med Virol, 65*(4), 751-757. doi:10.1002/jmv.2100
Hendrix, K. S., Meslin, E. M., Carroll, A. E., & Downs, S. M. (2013). Attitudes about the use of newborn dried blood spots for research: a survey of underrepresented parents. *Acad Pediatr, 13*(5), 451-457. doi:10.1016/j.acap.2013.04.010
Herida, M., Dervaux, B., & Desenclos, J. C. (2016). Economic Evaluations of Public Health Surveillance Systems: a Systematic Review. *Eur J Public Health, 26*(4), 674-680. doi:10.1093/eurpub/ckv250
Ibrahim, S. A., Abdallah, A., Saleh, E. A., Osterhaus, A. D., & De Swart, R. L. (2006). Measles virus-specific antibody levels in Sudanese infants: a prospective study using filter-paper blood samples. *Epidemiol Infect, 134*(1), 79-85. doi:10.1017/S0950268805004620
Itell, H. L., McGuire, E. P., Muresan, P., Cunningham, C. K., McFarland, E. J., Borkowsky, W., . . . Fouda, G. G. (2018). Development and application of a multiplex assay for the simultaneous measurement of antibody responses elicited by common childhood vaccines. *Vaccine, 36*(37), 5600-5608. doi:10.1016/j.vaccine.2018.07.048
Keogh, E. (2005). Developing a framework for budgeting for household surveys in developing countries. In *Household surveys in developnig and transition countries: design, implementation and analysis* (pp. 279-300). New York, NY: United Nations.
Kilic, T. S., Umar; Uematsu, Hiroki; Yoshida, Nobuo. (2017). *Costing household surveys for monitoring progress toward ending extreme poverty and boosting shared prosperity*. Washington, DC. http://documents.worldbank.org/curated/en/260501485264312208/Costing-household-

surveys-for-monitoring-progress-toward-ending-extreme-poverty-and-boosting-shared-prosperity
Kish, L. (1965). *Survey sampling*. New York,: J. Wiley.
Klein, S. L., Marriott, I., & Fish, E. N. (2015). Sex-based differences in immune function and responses to vaccination. *Trans R Soc Trop Med Hyg, 109*(1), 9-15. doi:10.1093/trstmh/tru167
Knight, L., Makusha, T., Lim, J., Peck, R., Taegtmeyer, M., & van Rooyen, H. (2017). "I think it is right": a qualitative exploration of the acceptability and desired future use of oral swab and finger-prick HIV self-tests by lay users in KwaZulu-Natal, South Africa. *BMC Res Notes, 10*(1), 486. doi:10.1186/s13104-017-2810-7
Kolawole, O. M., Anjorin, E. O., Adekanle, D. A., Kolawole, C. F., & Durowade, K. A. (2014). Seroprevalence of rubella IgG antibody in pregnant women in osogbo, Nigeria. *Int J Prev Med, 5*(3), 287-292.
Kombich JJ, M. P., Borus PK. (2012). Seroprevalence of Natural Rubella Antibodies among Antenatal Attendees at Moi Teaching and Referral Hospital, Eldoret, Kenya. *Journal of Immunological Techniques in Infectious Diseases, 1*(1). doi:http://dx.doi.org/10.4172/2325-9752.1000102
Lanieri T, R. S., Abernathy E and Icenogle J. (2008). Chapter 14: Rubella. In CDC (Ed.), *Manual for the surveillance of vaccine-preventable diseases* (6 ed.). Atlanta, GA.
Larsen, D. A., Chisha, Z., Winters, B., Mwanza, M., Kamuliwo, M., Mbwili, C., . . . Winters, A. M. (2015). Malaria surveillance in low-transmission areas of Zambia using reactive case detection. *Malar J, 14*, 465. doi:10.1186/s12936-015-0895-9
Lessler, J., & Metcalf, C. J. (2013). Balancing evidence and uncertainty when considering rubella vaccine introduction. *PLoS One, 8*(7), e67639. doi:10.1371/journal.pone.0067639
Lessler, J., Metcalf, C. J., Cutts, F. T., & Grenfell, B. T. (2016). Impact on Epidemic Measles of Vaccination Campaigns Triggered by Disease Outbreaks or Serosurveys: A Modeling Study. *PLoS Med, 13*(10), e1002144. doi:10.1371/journal.pmed.1002144
Lessler, J., Metcalf, C. J., Grais, R. F., Luquero, F. J., Cummings, D. A., & Grenfell, B. T. (2011). Measuring the performance of vaccination programs using cross-sectional surveys: a likelihood framework and retrospective analysis. *PLoS Med, 8*(10), e1001110. doi:10.1371/journal.pmed.1001110
Levin, H. M., & McEwan, P. J. (2001). *Cost-effectiveness analysis : methods and applications* (2nd ed.). Thousand Oaks, Calif.: Sage Publications.
Libman, M. D., Behr, M. A., Martel, N., & Ward, B. J. (2000). Rubella susceptibility predicts measles susceptibility: implications for postpartum immunization. *Clin Infect Dis, 31*(6), 1501-1503. doi:10.1086/317481
Liu, L., Li, M., Yang, L., Ju, L., Tan, B., Walker, N., . . . Guo, Y. (2013). Measuring coverage in MNCH: a validation study linking population survey derived coverage to maternal, newborn, and child health care records in rural China. *PLoS One, 8*(5), e60762. doi:10.1371/journal.pone.0060762
Loevinsohn, G., Rosman, L., & Moss, W. J. (2019). Measles Seroprevalence and Vaccine Responses in Human Immunodeficiency Virus-infected Adolescents and Adults: A Systematic Review. *Clin Infect Dis, 69*(5), 836-844. doi:10.1093/cid/ciy980
Lugner, A. K., Mollema, L., Ruijs, W. L., & Hahne, S. J. (2010). A cost-utility analysis of antenatal screening to prevent congenital rubella syndrome. *Epidemiol Infect, 138*(8), 1172-1184. doi:10.1017/S0950268809991336

Luman, E. T., Worku, A., Berhane, Y., Martin, R., & Cairns, L. (2007). Comparison of two survey methodologies to assess vaccination coverage. *Int J Epidemiol, 36*(3), 633-641. doi:10.1093/ije/dym025

Machekanyanga, Z., Ndiaye, S., Gerede, R., Chindedza, K., Chigodo, C., Shibeshi, M. E., . . . Kaiser, R. (2017). Qualitative Assessment of Vaccination Hesitancy Among Members of the Apostolic Church of Zimbabwe: A Case Study. *J Relig Health, 56*(5), 1683-1691. doi:10.1007/s10943-017-0428-7

MacNeil, A., Lee, C. W., & Dietz, V. (2014). Issues and considerations in the use of serologic biomarkers for classifying vaccination history in household surveys. *Vaccine, 32*(39), 4893-4900. doi:10.1016/j.vaccine.2014.07.005

Masresha, B. G., Dixon, M. G., Kriss, J. L., Katsande, R., Shibeshi, M. E., Luce, R., . . . Mihigo, R. (2017). Progress Toward Measles Elimination - African Region, 2013-2016. *MMWR Morb Mortal Wkly Rep, 66*(17), 436-443. doi:10.15585/mmwr.mm6617a2

Masresha, B. G., Kaiser, R., Eshetu, M., Katsande, R., Luce, R., Fall, A., . . . Prevention. (2014). Progress toward measles preelimination--African Region, 2011-2012. *MMWR Morb Mortal Wkly Rep, 63*(13), 285-291.

Mazaba-Liwewe ML, M. M., Babaniyi OO, Siziya S, Phiri D, Mulenga J, Michelo C. (2015). Sero-prevalence of rubella-specific immunoglobulin G antibodies and its correlates among blood donors in Zambia. *International Journal of Public Health, 7*, 321-330.

Mazaba, M., I., N., J., C., Msiska, T., Mutambo, H., Micheelo, C., . . . Monze, M. (2012). *Rubella amongst suspected measles cases under the EPI surveillance program in Zambia (2004-2011)*. Paper presented at the International Congress on Infectious Diseases, Thailand. http://www.ijidonline.com/article/S1201-9712(12)00462-6/abstract

Mazaba, M. L., Monze, M., Babaniyi, O. A., Siziya, S., & Michelo, C. (2015). Characterization of rubella seronegative females in the zambian blood donor community. *Front Public Health, 3*, 59. doi:10.3389/fpubh.2015.00059

Mazaba, M. L., Mtaja, A., Chabala, C., Sinyangwe, S., Babaniyi, O., Monze, M., . . . Michelo, C. (2014). Laboratory-confirmed Congenital Rubella Syndrome at the University Teaching Hospital in Lusaka, Zambia-Case Reports. *Medical Journal of Zambia, 41*(1), 24-31.

McEwan, P. J. (2012). Cost-effectiveness analysis of education and health interventions in developing countries. *Journal of Development Effectiveness, 4*(2), 189-213.

McLeroy, K. R., Bibeau, D., Steckler, A., & Glanz, K. (1988). An ecological perspective on health promotion programs. *Health Educ Q, 15*(4), 351-377.

Mehtani, N. J., Rosman, L., & Moss, W. J. (2019). Immunogenicity and Safety of Measles Vaccine in HIV-infected Children: an Updated Systematic Review. *Am J Epidemiol*. doi:10.1093/aje/kwz144

Mei, J. V., Alexander, J. R., Adam, B. W., & Hannon, W. H. (2001). Use of filter paper for the collection and analysis of human whole blood specimens. *J Nutr, 131*(5), 1631S-1636S.

Mercader, S., Featherstone, D., & Bellini, W. J. (2006). Comparison of available methods to elute serum from dried blood spot samples for measles serology. *J Virol Methods, 137*(1), 140-149. doi:10.1016/j.jviromet.2006.06.018

Metcalf, C. J., Farrar, J., Cutts, F. T., Basta, N. E., Graham, A. L., Lessler, J., . . . Grenfell, B. T. (2016). Use of serological surveys to generate key insights into the changing global landscape of infectious disease. *Lancet, 388*(10045), 728-730. doi:10.1016/S0140-6736(16)30164-7

Mirambo, M. M., Majigo, M., Aboud, S., Gross, U., & Mshana, S. E. (2015). Serological makers of rubella infection in Africa in the pre vaccination era: a systematic review. *BMC Res Notes, 8*, 716. doi:10.1186/s13104-015-1711-x

Mitchell, L. A. (1999). Sex differences in antibody- and cell-mediated immune response to rubella re-immunisation. *J Med Microbiol, 48*(12), 1075-1080. doi:10.1099/00222615-48-12-1075

Moreno-John, G., Gachie, A., Fleming, C. M., Napoles-Springer, A., Mutran, E., Manson, S. M., & Perez-Stable, E. J. (2004). Ethnic minority older adults participating in clinical research: developing trust. *J Aging Health, 16*(5 Suppl), 93S-123S. doi:10.1177/0898264304268151

Mpabalwani ME, M. B., Katepa-Bwalya N, Mukonka V, Mutambo H, Babaniyi OA. (2013). The 2010-2011 measles outbreak in Zambia: Challenges and lessons learnt for future action. *East African Journal of Public Health, 10*(1).

Mutembo, S., Carcelen, A., Mwansa, F. D., Searle, K., Wanyiri, J. W., Book, C., . . . Hayford, K. (2018). Integrating Blood Collection within Household Surveys: Lessons Learned from Nesting a Measles and Rubella Serological Survey within a Post-Campaign Coverage Evaluation Survey in Southern Province, Zambia. *Am J Trop Med Hyg, 99*(6), 1639-1642. doi:10.4269/ajtmh.18-0320

Mwambe, B., Mirambo, M. M., Mshana, S. E., Massinde, A. N., Kidenya, B. R., Michael, D., . . . Gross, U. (2014). Sero-positivity rate of rubella and associated factors among pregnant women attending antenatal care in Mwanza, Tanzania. *BMC Pregnancy Childbirth, 14*, 95. doi:10.1186/1471-2393-14-95

Nakalembe, M., Mutyaba, T., & Mirembe, F. (2016). Acceptability of study procedures (self-collected introital swabs, blood draws and stool sample collection) by students 10-16 years for an HPV vaccine effectiveness study: a pilot study. *BMC Res Notes, 9*, 170. doi:10.1186/s13104-016-1984-8

Nardone, A., Tischer, A., Andrews, N., Backhouse, J., Theeten, H., Gatcheva, N., . . . Miller, E. (2008). Comparison of rubella seroepidemiology in 17 countries: progress towards international disease control targets. *Bull World Health Organ, 86*(2), 118-125.

Novello, F., Ridolfi, B., Fiore, L., Buttinelli, G., Medda, E., Favero, A., . . . Caglioppa, F. (1996). Comparison of capillary blood versus venous blood samples in the assessment of immunity to measles. *J Virol Methods, 61*(1-2), 73-77. doi:10.1016/0166-0934(96)02071-x

Nyaoke, B. A., Mutua, G. N., Sajabi, R., Nyasani, D., Mureithi, M. W., & Anzala, O. A. (2017). Volunteer motivators for participating in HIV vaccine clinical trials in Nairobi, Kenya. *PLoS One, 12*(9), e0183788. doi:10.1371/journal.pone.0183788

Ohuma, E. O., Okiro, E. A., Bett, A., Abwao, J., Were, S., Samuel, D., . . . Nokes, D. J. (2009). Evaluation of a measles vaccine campaign by oral-fluid surveys in a rural Kenyan district: interpretation of antibody prevalence data using mixture models. *Epidemiol Infect, 137*(2), 227-233. doi:10.1017/S0950268808000848

Okello, G., Ndegwa, S. N., Halliday, K. E., Hanson, K., Brooker, S. J., & Jones, C. (2012). Local perceptions of intermittent screening and treatment for malaria in school children on the south coast of Kenya. *Malar J, 11*, 185. doi:10.1186/1475-2875-11-185

Onakewhor, J. U., & Chiwuzie, J. (2011). Seroprevalence survey of rubella infection in pregnancy at the University of Benin Teaching Hospital, Benin City, Nigeria. *Niger J Clin Pract, 14*(2), 140-145. doi:10.4103/1119-3077.84002

Osborne, K., Gay, N., Hesketh, L., Morgan-Capner, P., & Miller, E. (2000). Ten years of serological surveillance in England and Wales: methods, results, implications and action. *Int J Epidemiol, 29*(2), 362-368.

Ostermann, J., Njau, B., Mtuy, T., Brown, D. S., Muhlbacher, A., & Thielman, N. (2015). One size does not fit all: HIV testing preferences differ among high-risk groups in Northern Tanzania. *AIDS Care, 27*(5), 595-603. doi:10.1080/09540121.2014.998612

Pan American Health Organization. (2016). Measles elimination in the Americas [Press release]. Retrieved from http://www.paho.org/hq/index.php?option=com_content&id=12526%3Ameasles-elimination-in-the-americas

Pan American Health Organization. (2018). The challenge of maintaining the elimination of measles in the Americas, 2017-2018. *Immunization Newsletter.* Retrieved from https://www.paho.org/hq/index.php?option=com_docman&view=download&category_slug=immunization-newsletter-9&alias=47300-immunization-newsletter-september-2018&Itemid=270&lang=en

Parker, S. P., & Cubitt, W. D. (1999). The use of the dried blood spot sample in epidemiological studies. *J Clin Pathol, 52*(9), 633-639.

Patel, M. (2016). Measles and Rubella Serosurveys.

Pettersson, H. a. S., Bounthavy. (2015). Cost model for an income and expenditure survey. In *Household sample surveys in developing and transition countires* (pp. 267-278). New York, NY: United Nations.

Plans-Rubio, P. (2014). Is the current prevention strategy based on vaccination coverage and epidemiological surveillance sufficient to achieve measles and rubella elimination in Europe? *Expert Rev Anti Infect Ther, 12*(7), 723-726. doi:10.1586/14787210.2014.917047

Plotkin, S. A. (2010). Correlates of protection induced by vaccination. *Clin Vaccine Immunol, 17*(7), 1055-1065. doi:10.1128/CVI.00131-10

Polonsky, J. A., Juan-Giner, A., Hurtado, N., Masiku, C., Kagoli, M., & Grais, R. F. (2015). Measles seroprevalence in Chiradzulu district, Malawi: Implications for evaluating vaccine coverage. *Vaccine, 33*(36), 4554-4558. doi:10.1016/j.vaccine.2015.07.007

Protiere, C., Spire, B., Mora, M., Poizot-Martin, I., Preau, M., Doumergue, M., . . . Suzan-Monti, M. (2017). Patterns of patient and healthcare provider viewpoints regarding participation in HIV cure-related clinical trials. Findings from a multicentre French survey using Q methodology (ANRS-APSEC). *PLoS One, 12*(11), e0187489. doi:10.1371/journal.pone.0187489

Punnarugsa, V., & Mungmee, V. (1991). Detection of rubella virus immunoglobulin G (IgG) and IgM antibodies in whole blood on Whatman paper: comparison with detection in sera. *J Clin Microbiol, 29*(10), 2209-2212.

Ratnam, S., West, R., Gadag, V., Williams, B., & Oates, E. (1997). Rubella antibody levels in school-aged children in Newfoundland: Implications for a two-dose rubella vaccination strategy. *Can J Infect Dis, 8*(2), 85-88. doi:10.1155/1997/215383

Reef, S. E., Strebel, P., Dabbagh, A., Gacic-Dobo, M., & Cochi, S. (2011). Progress toward control of rubella and prevention of congenital rubella syndrome--worldwide, 2009. *J Infect Dis, 204 Suppl 1*, S24-27. doi:10.1093/infdis/jir155

Salihu, H. M., Wilson, R. E., King, L. M., Marty, P. J., & Whiteman, V. E. (2015). Socio-ecological Model as a Framework for Overcoming Barriers and Challenges in

Randomized Control Trials in Minority and Underserved Communities. *Int J MCH AIDS, 3*(1), 85-95.
Schutte, C., Chansa, C., Marinda, E., Guthrie, T. A., Banda, S., Nombewu, Z., . . . Kinghorn, A. (2015). Cost analysis of routine immunisation in Zambia. *Vaccine, 33 Suppl 1*, A47-52. doi:10.1016/j.vaccine.2014.12.040
Scott, P., Moss, W. J., Gilani, Z., & Low, N. (2011). Measles vaccination in HIV-infected children: systematic review and meta-analysis of safety and immunogenicity. *J Infect Dis, 204 Suppl 1*, S164-178. doi:10.1093/infdis/jir071
Snijdewind, I. J., van Kampen, J. J., Fraaij, P. L., van der Ende, M. E., Osterhaus, A. D., & Gruters, R. A. (2012). Current and future applications of dried blood spots in viral disease management. *Antiviral Res, 93*(3), 309-321. doi:10.1016/j.antiviral.2011.12.011
Sutcliffe, C. G., Searle, K., Matakala, H. K., Greenman, M. P., Rainwater-Lovett, K., Thuma, P. E., & Moss, W. J. (2017). Measles and Rubella Seroprevalence Among HIV-infected and Uninfected Zambian Youth. *Pediatr Infect Dis J, 36*(3), 301-306. doi:10.1097/INF.0000000000001422
Tahita, M. C., Hubschen, J. M., Tarnagda, Z., Ernest, D., Charpentier, E., Kremer, J. R., . . . Ouedraogo, J. B. (2013). Rubella seroprevalence among pregnant women in Burkina Faso. *BMC Infect Dis, 13*, 164. doi:10.1186/1471-2334-13-164
Thomas, H. I., & Aird, H. C. (1999). Maintenance of high-avidity rubella-specific IgG antibody and titres in recent HIV seroconvertors and in patients progressing to the AIDS-related complex and AIDS. *J Med Virol, 58*(3), 273-279.
Thompson, K. M., & Odahowski, C. L. (2016). Systematic Review of Measles and Rubella Serology Studies. *Risk Anal, 36*(7), 1459-1486. doi:10.1111/risa.12430
Thompson, K. M., Odahowski, C. L., Goodson, J. L., Reef, S. E., & Perry, R. T. (2016). Synthesis of Evidence to Characterize National Measles and Rubella Exposure and Immunization Histories. *Risk Anal, 36*(7), 1427-1458. doi:10.1111/risa.12454
Tischer, A., Andrews, N., Kafatos, G., Nardone, A., Berbers, G., Davidkin, I., . . . Miller, E. (2007). Standardization of measles, mumps and rubella assays to enable comparisons of seroprevalence data across 21 European countries and Australia. *Epidemiol Infect, 135*(5), 787-797. doi:10.1017/S0950268807008266
Toscano, C. M., Vijayaraghavan, M., Salazar-Bolanos, H. M., Bolanos-Acuna, H. M., Ruiz-Gonzalez, A. I., Barrantes-Solis, T., . . . i, V. P. D. W. T. (2013). Cost analysis of an integrated vaccine-preventable disease surveillance system in Costa Rica. *Vaccine, 31 Suppl 3*, C88-93. doi:10.1016/j.vaccine.2013.05.036
Travassos, M. A., Beyene, B., Adam, Z., Campbell, J. D., Mulholland, N., Diarra, S. S., . . . Levine, M. M. (2015). Strategies for Coordination of a Serosurvey in Parallel with an Immunization Coverage Survey. *Am J Trop Med Hyg, 93*(2), 416-424. doi:10.4269/ajtmh.15-0198
Trentini, F., Poletti, P., Merler, S., & Melegaro, A. (2017). Measles immunity gaps and the progress towards elimination: a multi-country modelling analysis. *Lancet Infect Dis, 17*(10), 1089-1097. doi:10.1016/S1473-3099(17)30421-8
Tripepi, G., Jager, K. J., Dekker, F. W., & Zoccali, C. (2010). Stratification for confounding--part 2: direct and indirect standardization. *Nephron Clin Pract, 116*(4), c322-325. doi:10.1159/000319591

Turner, A. G., Magnani, R. J., & Shuaib, M. (1996). A not quite as quick but much cleaner alternative to the Expanded Programme on Immunization (EPI) Cluster Survey design. *Int J Epidemiol, 25*(1), 198-203.

UNICEF. (2011). *Levels & trends in child mortality report 2011*. Retrieved from https://data.unicef.org/wp-content/uploads/2015/12/Child_Mortality_Report_2011_49.pdf

UNICEF. (2013, 31 December 2013). Zambia Statistics. Retrieved from http://www.unicef.org/infobycountry/zambia_statistics.html

UNICEF. (2017, 4 July 2017). Zambia: WHO and UNICEF estimates of immunization coverage: 2016 revision. Retrieved from https://data.unicef.org/wp-content/uploads/country_profiles/Zambia/immunization_country_profiles/immunization_zmb.pdf.

Vojnov, L., Taegtmeyer, M., Boeke, C., Markby, J., Harris, L., Doherty, M., . . . Ford, N. (2019). Performance of non-laboratory staff for diagnostic testing and specimen collection in HIV programs: A systematic review and meta-analysis. *PLoS One, 14*(5), e0216277. doi:10.1371/journal.pone.0216277

Vynnycky, E., Adams, E. J., Cutts, F. T., Reef, S. E., Navar, A. M., Simons, E., . . . Dabbagh, A. J. (2016). Using Seroprevalence and Immunisation Coverage Data to Estimate the Global Burden of Congenital Rubella Syndrome, 1996-2010: A Systematic Review. *PLoS One, 11*(3), e0149160. doi:10.1371/journal.pone.0149160

Watts, T. (1983). Rubella antibodies in a sample of Lusaka mothers. *Med J Zambia, 17*(4), 109-110.

Welz, T., Hosegood, V., Jaffar, S., Batzing-Feigenbaum, J., Herbst, K., & Newell, M. L. (2007). Continued very high prevalence of HIV infection in rural KwaZulu-Natal, South Africa: a population-based longitudinal study. *AIDS, 21*(11), 1467-1472. doi:10.1097/QAD.0b013e3280ef6af2

Wilson, S. E., Deeks, S. L., Hatchette, T. F., & Crowcroft, N. S. (2012). The role of seroepidemiology in the comprehensive surveillance of vaccine-preventable diseases. *CMAJ, 184*(1), E70-76. doi:10.1503/cmaj.110506

Winter, A. K., Martinez, M. E., Cutts, F. T., Moss, W. J., Ferrari, M. J., McKee, A., . . . Metcalf, C. J. E. (2018). Benefits and Challenges in Using Seroprevalence Data to Inform Models for Measles and Rubella Elimination. *J Infect Dis, 218*(3), 355-364. doi:10.1093/infdis/jiy137

World Bank. (2018). Official exchange rate (LCU per US$, period average) - Zambia.

World Health Organization. (2010). WHO guidelines on drawing blood: best practices in phlebotomy. 125.

World Health Organization. (2012). *Global Measles and Rubella: Strategic Plan 2012-2020*. Retrieved from Switzerland: http://apps.who.int/iris/bitstream/10665/44855/1/9789241503396_eng.pdf

World Health Organization. (2015). *Guidelines on the use of Serosurveys in Support of Measles and Rubella Elimination*. Retrieved from Geneva, Switzerland: https://www.who.int/immunization/monitoring_surveillance/burden/laboratory/serosurvey/en/

World Health Organization. (2018a, April 2018). Summary tables of mortality estimates by cause, age and sex, by country, 2000–2016. Retrieved from https://www.who.int/healthinfo/global_burden_disease/estimates/en/

World Health Organization. (2018b). Vaccination Coverage Cluster Surveys: Refernce Manual.
World Health Organization. (2019). *Manual for the laboratory-based surveillance of measles, rubella, and congenital rubella syndrome*. Retrieved from https://www.who.int/immunization/monitoring_surveillance/burden/laboratory/manual/en/
World Health Organization Regional Office for the Wesetern Pacific. (2017). *Sixth annual meeting of the regional verification commission for measles elimination in the Western Pacific*. Retrieved from Manila, Philippines: https://apps.who.int/iris/bitstream/handle/10665/259629/RS-2017-GE-49-CHN-eng.pdf
Yansaneh, I. S. (2005). An analysis of cost issues for surveys in developing and transition countries. In *Household sample surveys in developing and transition countries* (pp. 253-266). New York, NY: United Nations.
Zambia Central Statistical Office. (2012). *2010 Census of Population and Housing*. Retrieved from Lusaka, Zambia: https://www.zamstats.gov.zm/phocadownload/2010_Census/2010%20Census%20of%20Population%20National%20Analytical%20Report.pdf
Zambia Central Statistical Office. (2013). *Population and Demographic Projections 2011 - 2035*. Retrieved from Lusaka, Zambia: https://www.zamstats.gov.zm/index.php/publications/category/55-census-projections
Zambia Central Statistical Office. (2014). *Zambia Demographic and Health Survey, 2013-14*. Retrieved from Lusaka, Zambia: https://dhsprogram.com/pubs/pdf/PR53/PR53.pdf
Zambian Ministry of Health. (2017). *Post MR Campaign Survey Report*. Retrieved from
Zambian Ministry of Health. (2019a). *Case-based surveillance data*.
Zambian Ministry of Health. (2019b). *Health Management Information System*.
Zionism. (2019). In E. o. E. Britannica (Ed.), *Encyclopedia Britannica*. Online: Encyclopaedia Britannica, Inc.

Curriculum Vitae

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EDUCATION AND TRAINING

Degree	Year	Institution	Field
PhD	2019	Johns Hopkins Bloomberg School of Public Health	International Health -Concentration in Global Disease Epidemiology and Control -GPA: 4.0
MPH	2010	University of Alabama at Birmingham School of Public Health	Maternal and Child Health -GPA: 4.0
BA	2008	George Washington University	International Affairs and French Language -Concentration in Global Public Health -Magna Cum Laude
Study abroad	2007	Nanterre University	French Language and Literature

PROFESIONAL EXPERIENCE

Position	Dates	Institution
Doctoral student researcher	March 2016-present	Johns Hopkins University, Bloomberg School of Public Health, International Vaccine Access Center

Primary responsibilities included:

- Conduct a nested serosurvey as part of a post-campaign coverage survey in Zambia
- Conduct costing analysis for serosurvey implementation
- Conduct qualitative research on acceptability of serosurvey implementation
- Propose design strategies for serological surveillance in India and Zambia

Position	Dates	Institution
Doctoral student researcher	August 2017-July 2018	Johns Hopkins University, Bloomberg School of Public Health, International Health Department

National Institute for Allergy & Infectious Diseases awarded a supplemental grant for maternal immunization. Primary responsibilities included:

- Supported multi-country project on maternal immunization in Latin America
- Collaborated with Pan American Health Organization for fieldwork
- Conducted independent qualitative research among pregnant women at field site in Peru

Position	Dates	Institution
Doctoral student researcher	October 2015-February 2016	Johns Hopkins University, Bloomberg School of Public Health, International Vaccine Access Center

Primary responsibilities included:

- Developed standard operating procedures and training materials for vaccination coverage surveys in Pakistan
- Create database, entry screens, and data cleaning procedures for monitoring incoming data

Position	Dates	Institution
Public health advisor	August 2012- September 2015	Centers for Disease Control, Center for Global Health, Disease Eradication and Elimination Branch

Primary responsibilities included:

Monitoring and evaluation

- Served as external monitor for measles campaign in Cote d'Ivoire, noting the sensitivities within the context of an Ebola outbreak in neighboring countries
- Conducted multi-country evaluation of Stop the Transmission of Polio training program
- Developed and synthesized of materials for the 4th annual western pacific regional measles verification committee and composed the final report
- Provided technical assistance to WHO-Jordan for measles outbreak response campaign through independent monitoring, supervision and analysis of rapid convenience assessments, and coordination of post-campaign coverage survey training, and data entry

Surveillance and outbreak response

- Responded to Ebola outbreak in Nigeria to set up Emergency Operations Center and Ebola treatment center in Lagos which resulted in containment of the outbreak
- Managed a study to investigate ebolavirus infections among healthcare workers in Guinea and provided technical support to the sub-national ministry of health to conduct outbreak investigations and address infection prevention and control issues identified
- Analyzed measles surveillance data using a newly developed risk assessment tool and created a risk profile for districts & states in Sudan, Cote d'Ivoire and Tanzania to help guide measles elimination activities
- Conducted 6-month post-epidemic poliovirus review and rapid Acute Flaccid Paralysis surveillance review in Democratic Republic of Congo

New vaccine introduction

- Conducted Inactivated Polio Vaccine (IPV) acceptability study in Nigeria in preparation for introduction of IPV. Sits on national IPV introduction working group.
- Supported cluster survey for Measles-Rubella-HPV campaign and Expanded Program for Immunization in Rwanda by developing protocol, editing questionnaires, training interviewers, monitoring data collection, developing data entry screens, training data enterers, and cleaning data
- Assisted with coordination for the introduction of pentavalent vaccine into the routine immunization program in Haiti

Capacity development

- Supported routine immunization strengthening in Nigeria through training of National Stop Transmission of Polio officers, new vaccine introduction working group, and information systems strengthening
- Provided technical assistance for field epidemiology training programs to further global immunization goals in Honduras and Brazil

Grant Management

- Managed 2013-18 financial resource requirement budgeting for the Measles-Rubella Initiative, a partnership between CDC, WHO, UNICEF, and UNF
- Collaborated with grantees to support management of multi-million-dollar cooperative agreements with WHO, UNICEF, UNF, and KidRisk
- Provided technical input for the development of global health security funding proposals for countries to support vaccine preventable disease activities

Position	Dates	Institution
Public health advisor	August 2010-July 2012	Centers for Disease Control, National Center for Immunization and Respiratory Diseases

Primary responsibilities included:

- Conducted research on billing in the public health sector and aided with the implementation of funding to build capacitate at health departments to bill for immunizations
- Drafted USG Position paper on global immunization and vaccine preventable disease topics for the World Health Assembly
- Appointed as Policy Unit Lead in the Emergency Operations Center's for Polio Response and Historian for Haiti Cholera Response
- Developed materials for internal and external partners including Congressional, Executive and international administration level documents on CDC's immunization and vaccine preventable disease activities
- Recruited and trained international participants for Stop the Transmission of Polio program
- Facilitated global evaluation of the Stop the Transmission of Polio program, a training program in vaccines for public health professionals
- Attended Advisory Committee on Immunization Practice meetings

Position	Dates	Institution
Research assistant	August 2008-May 2010	University of Alabama at Birmingham, Department of Preventive Medicine

Primary responsibilities included:

- Conducted research for three separate grants on breast and cervical cancer prevention as well as HPV awareness and nutrition promotion among Latino immigrant women
- Responsible for database management of long-term study, including data entry, cleaning, and analysis using SPSS
- Moderated focus groups of Latino women on discrimination in the health care setting
- Interviewed subjects in a poorly resourced community
- Conducted literature reviews on HPV, cervical cancer, vaccination uptake, and attitudes towards health among minority populations
- Disseminated information by writing newsletters, appearing on radio shows, and organizing health fairs for a Hispanic population about health issues and prevention strategies
- Organized a community workshop for cancer survivors to discuss legal and financial issues

PUBLICATIONS

Journal articles-published peer reviewed:

1. Hayford KH, Mutembo S, **Carcelen A**, et al. Measles and rubella serosurvey identifies rubella immunity gap in young adults of childbearing age in Zambia: The added value of nesting a serological survey within a post-campaign coverage evaluation survey. *Vaccine*. 2019 Apr 17;37(17):2387-2393.
2. Mutembo S, **Carcelen A**, et al. Integrating Blood Collection within Household Surveys: Lessons Learned from Nesting a Measles and Rubella Serological Survey within a Post-Campaign

Coverage Evaluation Survey in Southern Province, Zambia. *Am J Trop Med Hyg*. 2018 Dec; 99(6): 1639–1642.

3. Peralta-Carcelen M, Schwartz J, **Carcelen AC**. Behavioral and Socioemotional Development in Preterm Children. *Clin Perinatol*. 2018 Sep;45(3):529-546.
4. Lessler J, Chaisson LH, Kucirka LM, Bi Q, Grantz K, Salje H, **Carcelen AC**, et al. Assessing the global threat from Zika virus. *Science*. 2016 Aug 12;353.
5. Rico A, Brody D, Coronado F, Rondy M, Fiebig L, **Carcelen A**, et al. "Epidemiology of epidemic Ebola virus disease in Conkary and surrounding prefectures, Guinea 2014-2015." *Emerging Infectious Diseases*. 2016: (22)2.
6. Lessler J, Ott C, Chaisson L, **Carcelen A** et al. "Three key distributions of Zika infection and their implications: A Systematic Review and Pooled Analysis." *Bulletin of the World Health Organization* 2016; 94:841-849.

POSTERS AND PRESENTATIONS

Scientific meetings:

1. Attitudes towards maternal vaccination in Peru. *American Society of Tropical Medicine and Hygiene Conference 2018*. Poster presentation
2. Serological survey to monitor population immunity to measles and rubella viruses after a national measles-rubella vaccination campaign in Zambia. *American Society of Tropical Medicine and Hygiene Conference 2017*. Poster presentation
3. Serosurvey acceptability in Southern province, Zambia. *Vaccine Day 2018*. Poster presentation
4. CDC 2010 Haiti Cholera Response: Incident Management System's Policy Unit Challenges & Strategies. *American Public Health Association Conference 2011*. Poster presentation

PROFESSIONAL ACTIVITIES

Peer review activities

- PLOS ONE reviewer, February 2016

Consultations

- Centers for Disease Control and Prevention, Polio Legacy Planning, August 2016
- United States Agency for International Development, Health Policy Initiatives, July-August 2008

Review boards

- Subject matter expert for CDC objective review panel, 2011

HONORS AND AWARDS

Honors:

- Executive Board Member of Delta Omega Honor Society—Alpha Chapter
- CDC Center for Global Health outbreak response

Awards:

- 2019- Nancy Stephens Award at Johns Hopkins University
- 2019- David and Elinor Bodian Award at Johns Hopkins University
- 2016- Clements-Mann vaccine award at Johns Hopkins University
- 2015-17- Presidential Management Fellow with the United States Government
- 2008- Robert P Ginter Award at University of Alabama at Birmingham

Funding awards:

- 2017- Diversity Supplement Awardee from National Institute for Allergy and Infectious Diseases
- 2018- Graduate Student Research grant from Organization of Autism Research
- 2017- A Healthier World Planning Grant—graduate student researcher
- 2016- Wendy Klag—graduate student researcher

TEACHING

Course	No. credits	Dates	Department, University
Large-scale effectiveness evaluations of health programs	4	2019, 2017	International Health, JHSPH
Child and public health in the tropics	4	2019, 2017	International Health, JHSPH
Advances in community-oriented primary health care	4	2019	International Health, JHSPH
Vector-borne diseases in the tropics	4	2019	International Health, JHSPH
Global disease and control programs and policies	4	2018	International Health, JHSPH
Health information systems	3	2017	International Health, JHSPH
Research Methods & Needs Assessment and Evaluation	4	2010	Maternal and Child Health, UAB